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Investigating Risk Factors for Pediatric Opioid Morbidity and Mortality

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Pharmacology and Toxicology

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INVESTIGATING RISK FACTORS FOR PEDIATRIC OPIOID MORBIDITY AND
MORTALITY
(Thesis format: Integrated Article)

by

Lauren E Kelly

Graduate Program in Pharmacology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract and Keywords

Young children are sometimes prescribed opioids and may be exposed to opioids *in utero* and through breast milk. Clinical and genetic factors create large inter-individual variability in opioid response and have been associated with life threatening and often fatal adverse drug reactions in young children. Genetic factors have been studied in adults but there is little clinical evidence in young children. The focus of this thesis is on three commonly prescribed opioids: codeine, morphine and methadone. The objective of this work was to investigate risk factors associated with opioid related morbidity and mortality in young children. Risk factors were examined in three populations of children including neonates exposed to opioids *in utero*, infants exposed to codeine in breast milk as well as young children receiving codeine and morphine for post-surgical pain relief. We hypothesize that genetics and clinical factors will affect opioid response in young children.

As the prevalence of opioid use increases it is important to investigate clinical and genetic risk factors as well as cost-effective treatment options. Neonates exposed to opioids *in utero* do not show an increased risk for mortality. Genetics may play a role in the development of neonatal withdrawal symptoms following *in utero* methadone exposure. Further work is necessary in order to corroborate the role of genetic and clinical factors in predicting neonatal abstinence syndrome. Codeine use during lactation has been shown to result in a significant neonatal sedation, much of which was associated with maternal genotype and dose. Guidelines based on predetermined clinical risk factors were able to mitigate the previously identified increased genetic risk. Several fatalities have been reported following codeine use in children post-tonsillectomy. In a randomized clinical trial we found that standard morphine doses (0.2-0.5mg/kg) may not be a safe alternative in children with obstructive sleep apnea. The safety and effectiveness of lower morphine doses should be investigated.

Genetic variability in drug metabolizing enzymes, drug transporters and receptors, influence opioid response and create risks for adverse effects in young children. Standard doses of opioids are not safe in all children, and should be dosed on an individual basis.

Keywords: codeine, morphine, methadone, pharmacogenomics, adverse drug reactions, pediatrics

Co-Authorship Statement

Chapter 1: Introduction and Rationale

This manuscript was critically reviewed by Drs. Gideon Koren and Michael Rieder who provided guidance throughout the submission process. Dr. Parvaz Madadi revised and provided data for the included manuscript.

Chapter 2: In utero methadone exposure, neonatal abstinence syndrome and oral morphine weaning

Mrs. Karen Bridgman-Acker and Dr. Albert Lauwers were essential in identifying fatalities resulting from methadone exposure at the Chief Coroner's Office. Drs. Laura Lyons, Doreen Matsui and Henry Roukema along with David Knoppert, Nancy Watts and Shaylene Wong were responsible for enrolling expecting mothers and collecting blood and placenta samples. Lauren Kelly was responsible for data collection for all demographic and clinical variables as well as all data analysis. Dr. Roukema and Mr. Knoppert also assisted in identifying babes treated with oral morphine for neonatal abstinence syndrome. Methadone levels were quantified by Dr. David Freeman and Dr. Rommel Tirona. Genotyping was completed by Dr. Ute Schwartz. Drs. Koren, Rieder and Madadi participated in reviewing and editing the published manuscript.

Chapter 3: Neonatal safety of codeine in breast milk

Drs. Shahnaz Chadhoury, Geert 'tgong, Andrea Lausman and Howard Burger played a role in patient recruitment and obtaining informed consent. Lauren Kelly collected all chart data, completed patient follow-ups and analyzed all data. Dr. Parvaz Madadi and Myla Moretti participated in study design. Drs. Colin Ross, Michael Hayden and Bruce Carleton provided support with genotyping. Drs. Gideon Koren and Michael Rieder provided clinical expertise and critically reviewed this manuscript.

Chapter 4: Post-operative opioid use in children with obstructive sleep apnea

Drs. Gideon Koren and Michael Rieder provided insight regarding the case report and manuscript submission. Drs. Doron Sommer, Diane Reid, Jayant Ramakrishna and Jonathan Maclean provided Otolaryngology expertise, aided in study design and performed tonsillectomies. Lauren Kelly designed the study protocol, drafted the grant and all study documentation. Lauren Kelly created the data collection forms and analyzed all data. Stephanie Hoffbauer and Sadaf Arbabtafti were valuable research assistants, responsible for obtaining informed consent and data acquisition. Drs. Gideon Koren and Doron Sommer critically appraised and contributed to the manuscript.

Chapter 5: Overall perspective and future directions

David Knoppert and Henry Roukema assisted in the design of the study “In home oral morphine weaning vs in hospital”. Lauren Kelly reviewed all patient charts for inclusion, collected all data and completed the analysis. Drs. Gideon Koren, Michael Rieder and Henry Roukema along with David Knoppert aided in the design of a prospective clinical trial assessing the effectiveness of at home oral morphine weaning and pharmacogenomic predictors of NAS.

*All manuscripts in this thesis were written by Lauren E Kelly

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List of Abbreviations

- AAG- acidic alpha-1 globulin
- ADR – adverse drug reaction
- AHI – apnea hypopnea index
- AMP – adenosine monophosphate
- AS – activity score
- AT - adenotonsillectomy
- AUC – area-under-the-curve
- C6G – codeine-6-glucuronide
- cAMP – cyclic adenosine monophosphate
- CAS – Children’s Aid Society
- CIHI – Canadian Institute of Health Informatics
- CNS – central nervous system
- COMT – catechol-o-methyltransferase
- CREB – cyclic adenosine monophosphate response element binding protein
- CYP – cytochrome p 450
- EDDP - 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
- EMDP - 2-ethyl-5-methyl-3,3-diphenylpyrroline
- FDA – Federal Drug Administration
- GA – gestational age
- GDP – guanosine – 5'-diphosphate
- GI – gastrointestinal
- GLUT – glucose transporter
- GTP - guanosine – 5'-triphosphate
- IMR – infant mortality rate
- LOF – loss of function

LOS – length of stay
M3G – morphine-3-glucuronide
M6G – morphine-6-glucuronide
MAF – minor allele frequency
MMT – methadone maintenance treatment
MOR – mu-opioid receptor
NAS – neonatal abstinence syndrome
NICU – neonatal intensive care unit
NMDAR – N-methyl-D-aspartate receptor
NSAID – nonsteroidal anti-inflammatory drug
OATP – organic anion transporting polypeptide
OCC – Office of the Chief Coroner of Ontario
OCT – organic cation transporter
OPS – objective pain scale
OSA(S) – obstructive sleep apnea (syndrome)
P-gp – P-glycoprotein
PRN – pro re nata
PM – poor metaboliser
SDB – sleep disordered breathing
SNP- single nucleotide polymorphism
SUDS – sudden unexpected death syndrome
SUDI – sudden unexpected death in infancy
UGT – Uridine 5'-diphospho-glucuronosyltransferase
UM – ultra-rapid metaboliser

Chapter 1: Background and rationale

Part of this chapter has been adapted from published work:

Kelly L.E, Madadi P. Is there a role for Therapeutic Drug Monitoring with Codeine? *Therapeutic Drug Monitoring* 2012; 34 (3): 249-256

1.1 Overview: Opioid Pharmacology

Opioids are derived from the opium poppy, *Papaver somniferum* and are among the oldest known classes of drugs cultivated as early as 3400 BC (1). Morphine, the prototypical opioid, was isolated in 1804 by a German pharmacist and named after the God of dreams “morphium” (1). Morphine is one of four plant-derived amines that can be extracted from the opium poppy (along with codeine, thebaine and papavarine). Codeine was isolated in 1832, shortly after morphine (2). The term opioid is used to describe any morphine-like substance that acts on the opioid receptors and has activity that can be antagonized by naloxone. Opioids exist as naturally occurring compounds (morphine and codeine), semisynthetic compounds (oxycodone), as well as fully synthetic, designer opioids (methadone). Opioids are primarily used as analgesics and in children are indicated for use in sickle cell pain crises, cancer and postoperative pain. This thesis will focus on the clinically-important sources of variability in response of young children to three opioids; codeine, morphine and methadone.

Morphine:

Morphine, the archetypal opioid, is isolated from the seed of the opium poppy. Figure 1.1 shows the chemical structure of morphine which consists of a benzene ring with a phenolic hydroxyl group at binding site 3, nitrogen at position 5, and an alcohol hydroxyl group at position 6. Many natural and semisynthetic opioids are formed by changes in one or more of these three functional groups. For example, heroin (diacetylmorphine) is the product of O-acetylation at positions 3 and 6 (1). The tertiary nitrogen functional group is essential for morphine analgesia, as a quaternary nitrogen group is charged and too large to pass freely into

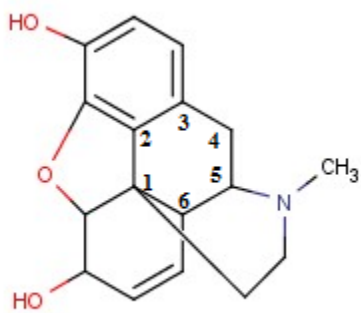
the central nervous system (CNS). This tertiary nitrogen group also makes morphine a weak base, with a pKa of 8. Morphine acts primarily through the mu-opioid receptor (MOR). These receptors, found in the brain, spinal cord, and intestinal tract, mediate both the therapeutic and adverse effects of morphine and other members of the opioid family. Medically, the main therapeutic benefit of morphine is analgesia, while adverse effects range from GI disturbances to respiratory depression. There are two MOR subtypes: Mu 1 is primarily responsible for the analgesic effects, whereas Mu 2 generally causes sedation, respiratory depression, euphoria, urinary retention, anorexia, pruritus, and dependence (1).

Morphine can be administered by a variety of different routes with oral and intravenous routes being most common. As with all drugs, the route and dose of administration will determine the maximum plasma concentration and at what time this will be reached. Morphine has elimination half-life of approximately 2 hours in infants and children (1, 3). Resulting from redistribution from peripheral tissue and enterohepatic recirculation a long terminal half-life has been reported following oral administration (4). The oral bioavailability of morphine is approximately 40% resulting from extensive first pass metabolism, and rapid conjugation with glucuronic acid in the gut. Morphine pharmacokinetic parameters in neonates and young children vary when compared to those of the well-studied adult population. The volume of distribution of morphine has been shown to increase exponentially with postnatal age (5). Clearance in infants and young children is estimated at 23.6 ± 8.5 ml/min/kg and reaches roughly 80% of adult morphine clearance by 6 months of age (5).

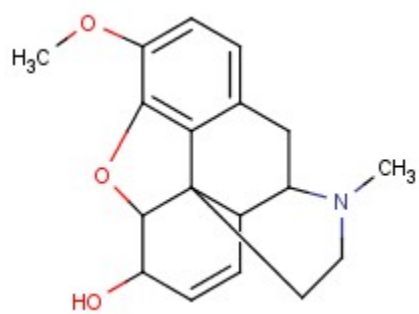
Morphine is glucuronidated by uridine diphospho glucuronosyltransferase (UGT) 2B7 and UGT 1A2 into morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). A small proportion of morphine (0-5%) is demethylated by cytochrome p 450 (CYP) 3A4 into normorphine. The M3G metabolite is primarily formed via UGT1A2 and is not active at the opioid receptor. Studies suggest that M3G may cause neuroexcitability at high concentrations (6). The M6G metabolite has roughly a 50-fold higher potency for the MOR than the parent compound (7). Morphine is eliminated mostly in urine (90%) and bile. Roughly 10% of morphine is eliminated unchanged while M3G is the major metabolite found in urine (8).

Morphine is approximately 50% ionized at physiologic pH allowing it to passively diffuse through tissue compartments within the body, including the blood brain barrier. Due to its relatively low lipid-solubility the penetration through the blood brain barrier is slow if administered via oral or intravenous routes. The M6G metabolite is more polar than morphine and studies suggest active transport of M6G into the CNS via the OATP 1A2 or GLUT-1 transporter (9, 10). Efflux of morphine out of the CNS is mediated by p-glycoprotein (11, 12). The ability of morphine to passively diffuse through cellular membranes results in transfer through the placenta and also into breast milk. Morphine is roughly 35% protein bound, leaving a large portion free to transfer through biological membranes. Morphine has a milk: plasma ratio of 1.1-3.6 and a relative infant dose of 9.1% (13). The relative infant dose characterizes how much of a mother's dose is likely to be found in breast milk. Since the clearance rate in the neonate is up to 5-fold slower than in the mother, neonatal exposure can be as high as 50% of that of the mother, which will be discussed in further detail later in this chapter.

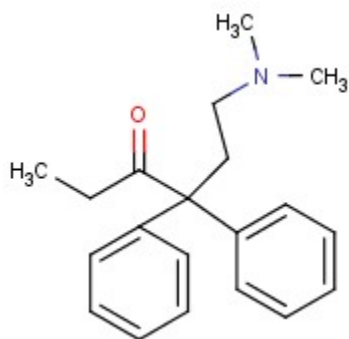
Figure 1.1. The chemical structures of morphine, codeine and methadone



Morphine



Codeine



Methadone

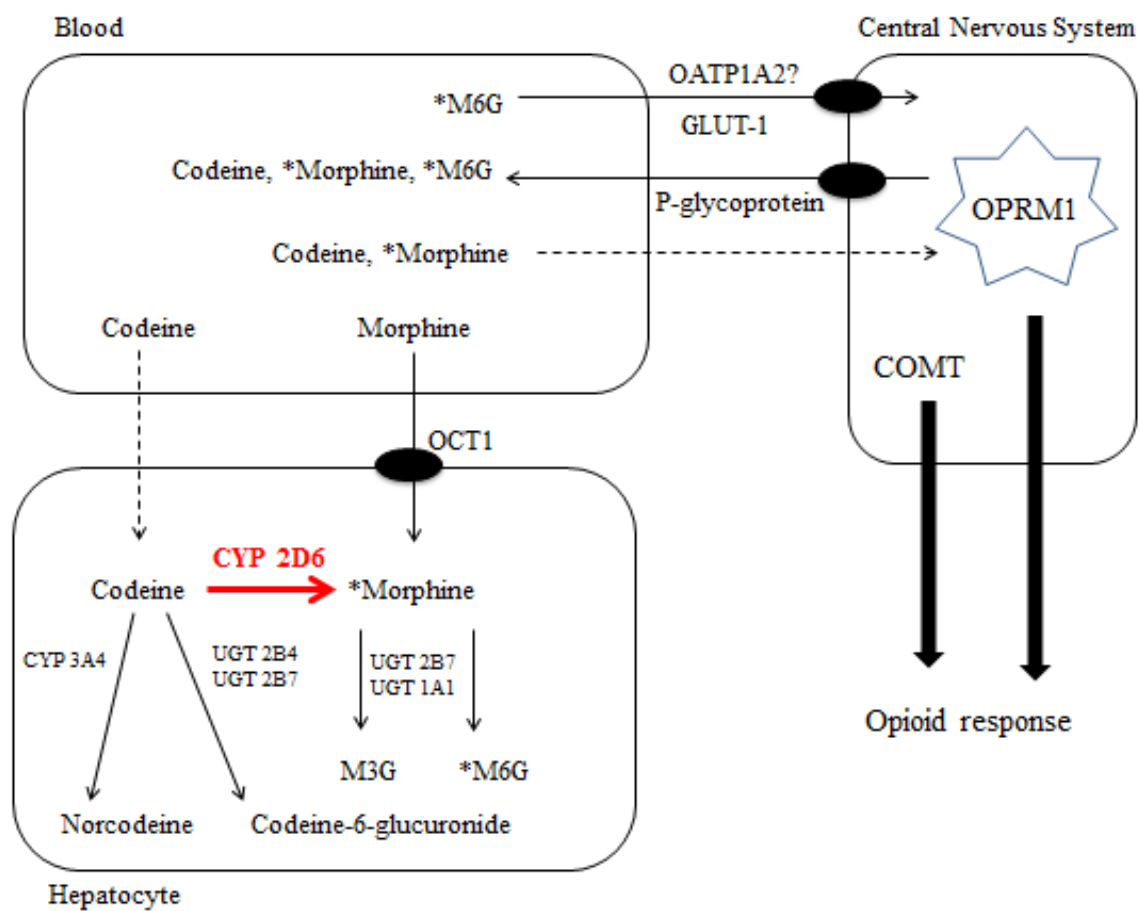
Codeine:

Second only to morphine, codeine is the most common opioid analgesic used to manage pain in children, frequently in combination with acetaminophen, marketed as Tylenol® 1 through 4 (14). The structure of codeine is nearly identical to that of morphine, with the addition of a methyl group to the phenolic group at position 3 (Figure 1.1). The methyl ether group in codeine (compared to the alcohol group in morphine) decreases polarity and is responsible for the high oral bioavailability of codeine (~ 90%) compared to morphine (~40%). The elimination half-life of codeine is roughly 2.5-3 hours and reaches its peak plasma concentration within 0.5-1.0 hours (1). Codeine metabolism is primarily hepatic, and the majority of codeine is glucuronidated by UGT2B4 and 2B7 to codeine-6-glucuronide (C6G) (Figure 1.2). The contribution of several unknown UGT isozymes to this pathway has been suggested. Codeine and its C6G metabolite have a low potency for the mu-opioid receptor (15). Codeine is also metabolized by cytochrome P 450 (CYP) 3A4 to norcodeine which is inactive.

A small proportion of codeine (5-15%) is bioactivated by CYP2D6 into morphine, which has a 200-fold greater affinity for the mu-opioid receptor. Hence, codeine is often considered a pro-drug as the majority of its analgesic effect is through its conversion to morphine in the liver. Similar to morphine, codeine undergoes passive diffusion and is actively effluxed by p-glycoprotein. Codeine crosses the placenta and has been shown to transfer into breast milk (16). Codeine has a milk plasma ratio of 1.3-2.5 and a relative infant dose of 8.1%. Of significance, the American Academy of Pediatrics generally considered codeine to be compatible with

breastfeeding until recently (13, 17). A fatal case of codeine toxicity in a breastfed neonate, which has changed this commonly –held view, will be discussed further in Chapter Three.

Figure 1.2. Schematic representation of the metabolism and transport of codeine and morphine in plasma, the hepatocyte and the central nervous system. Note: the diagram does not include codeine conversion to morphine via CYP2D6 in the CNS. Abbreviations used include: COMT = Catechol-O-methyltransferase, CYP = cytochrome p450, GLUT = glucose transporter, M6G = morphine-6-glucuronide, M3G = morphine-3-glucuronide, OATP = organic anion transporter protein, OPRM1= mu-opioid receptor, UGT = uridine diphospho glucuronosyltransferase



Methadone:

Methadone is a fully synthetic opioid agonist that was designed as an analgesic during the second world war, when morphine supplies were scarce (18). In North America, methadone is administered as a racemic mixture of equal parts (R)- and (S)-methadone. Methadone is lipophilic and generally well absorbed. The pharmacokinetic parameters of methadone are highly variable among individuals, with an oral bioavailability ranging from 36-100% and elimination half-life between 12 and 150 hours, with an average of 24 hours (1, 19-21). The long elimination half-life is due to its high liposolubility and redistribution from fat tissue. The dose of racemic methadone required to obtain a plasma methadone concentration of 250ng/ml in a 70kg patient taking no other medications can range from as low as 55 mg/day up to 921 mg/day (20) illustrating a large variability in dosing requirements. The peak plasma concentration of methadone is reached within 0.5-1 hour. The metabolic fate of methadone is shown in Figure 1.3. Methadone is primarily metabolized in the liver and intestine with several CYP enzymes responsible for inactivating the drug, primarily CYP3A4 and CYP2B6 (the latter, S-methadone stereospecific). Methadone metabolites are inactive and are eliminated in urine and in faeces and urine

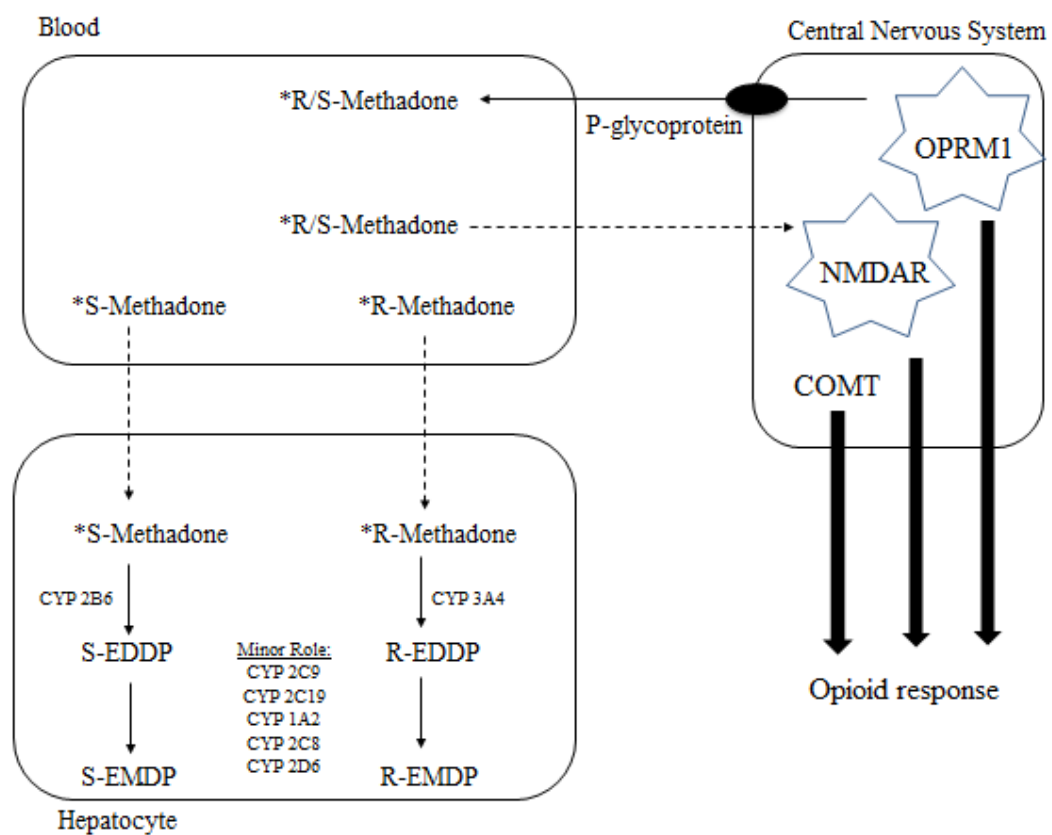
Methadone crosses the placenta (22) and low levels have been detected in breast milk (23). The low levels are likely due to the high percentage of methadone bound to the acidic- α_1 globulin (90%) that cannot passively diffuse through maternal capillaries. The milk/plasma ratio of methadone is 0.68 suggesting relatively low drug transfer, with the relative

infant dose between 1.9-6.5% (13). The American Academy of Pediatrics considers methadone compatible with breastfeeding at any maternal dose (17).

Methadone acts as an agonist at the MOR and an antagonist at the N-methyl-D-aspartate receptor (NMDAR). R-Methadone binds to the MOR with 10-fold higher potency than S-methadone which also acts at the NMDAR, decreasing glutaminergic transmission (24). NMDAR antagonism interferes with the neuroadaptive changes that maintain addictive behaviours. Although methadone is most frequently used to treat opioid dependency, its affordability, neuropathic pain potential, and long half-life have resulted in an increase in prescriptions for analgesia. The analgesic effects appear to last only 4 to 8 hours, and due to the long half-life of methadone, drug accumulation may occur with repeat dosing. S-Methadone also interacts with the human-ether-a-go-go voltage gated potassium channel in the heart, increasing the QT interval which has been associated with Torsades de Pointes (25). The risk for potentially fatal cardiac arrhythmia increases with methadone doses above 60mg/day, and conditions that affect the cardiac action potential such as hypokalemia (26).

Neonates exposed to methadone and /or other opioids *in utero* risk the development of neonatal abstinence syndrome (NAS) upon parturition. The withdrawal syndrome varies in severity and is characterized by disturbances to the central nervous system, metabolic and respiratory effects. Chapter Two will discuss the development and management of NAS in more detail.

Figure 1.3. Schematic representation of the metabolism and transport of methadone in plasma, the hepatocyte and the central nervous system. Abbreviations used include: COMT = Catechol-O-methyltransferase, CYP = cytochrome p450, EDDP = 2-ethylidene-1, 5-dimethyl-3,3-diphenylpyrrolidine, EMDP = 2-ethyl-5-methyl-3,3-diphenylpyrroline NMADR = N-methyl-D-aspartate receptor OPRM1= mu-opioid receptor.



1.2 Clinical sources of variability in pediatric response to opioids

A) Age:

Developmental age plays an important role in the ontogeny of the enzymes, transporter and target receptors involved in clearing opioids, especially the glucuronidation of morphine and subsequently morphine clearance. In children total morphine clearance is roughly 80% of adult values by 6 months of age and reaches 96% of adult levels by one year (5). Neonates have decreased fat and decreased muscle content compared to older children and adults. Neonates also have an immature glomerular filtration rate, especially if born premature, which can lead to accumulation of morphine metabolites that are primarily cleared in urine. Renal tubular secretion increases during the first few weeks of life and reaches adult levels by 8-12 months of age (27). The importance of age-dependent renal function to morphine clearance is evident as preterm neonates clear morphine at a rate of 2.2 ± 0.7 ml/min/kg compared to 8.1 ± 3.2 in term neonates and 23.6 ± 8.5 in infants and children (3). Glomerular filtration rates are low (~20% adults) for the first 24 hours of life and increase steadily for the first three months (28). Between the ages of 5-15 years the glomerular filtration rate is higher than in adults, resulting in an increased excretion rate of opioid metabolites in urine.

Increased oxygen consumption, decreased airway muscle control and an overall decrease in the ventilatory response to carbon dioxide (29) also increase the sensitivity of neonates and infants to the respiratory effects of opioids. The effects of opioids are generally prolonged in neonates as the development of hepatic drug metabolism enzymes matures at

variable rates, leading to reduced opioid metabolism. Conversely, children ages 2-6 years have been shown to have higher rates of metabolism via CYP enzymes, due to a larger liver mass per kg body weight, resulting in an increased opioid metabolism (normalized for weight) compared to adults (30).

B) Protein binding:

Circulating levels of plasma binding proteins determine the ratio of free to bound drug, and thus the amount of drug available to cross the blood brain barrier and elicit central pharmacological effects. Morphine is primarily bound to albumin (31) and albumin levels are only slightly lower than adult levels at birth (32). Methadone on the other hand is primarily bound to alpha₁ acidic glycoprotein (AAG). AAG expression has been shown to reach roughly 75% of adult levels by one year of age, but is only 25% at birth (28). The increased ratio of free: bound methadone will increase the potential for toxicity as methadone is a highly protein bound drug. Furthermore, drugs that compete for AAG binding can increase circulating levels of free methadone, thus enhancing the therapeutic and toxic effects. Plasma AAG levels have been shown to fluctuate significantly under stressful conditions such as heroin withdrawal (33) and in various disease states, and traumatic events (34, 35) which may alter the amount of free methadone in the neonate following *in utero* methadone exposure as well as exposure through breast milk.

C) Drug-Drug Interactions:

As many of the enzymes responsible for activating and deactivating codeine, morphine and methadone have broad substrate specificity, drug-drug interactions play a significant role in

opioid efficacy and toxicity. Drug-drug interactions will be discussed within the context of individual genes in the next section.

D) Health status:

Any health conditions that affect renal function or hepatic metabolism have the ability to alter opioid clearance in children. In adults, CYP enzyme hepatic oxidation is reduced in patients with severe liver disease resulting in a decreased drug clearance for opioids metabolized by these enzymes, such as methadone (36). Glucuronidation is affected to a much lesser extent than oxidation in cirrhosis patients, although the clearance of morphine was found to be decreased in some patients (36). Compared to healthy adults, patients with kidney failure have a greater accumulation of the analgesic (M6G) and neuroexcitatory (M3G) morphine metabolites, as well as an overall increase in the morphine area under curve (37). The altered morphine pharmacokinetics was reversed with a kidney transplant which may have important implications for children undergoing transplantation (37). Studies on the effects of hepatic and renal health status on morphine pharmacokinetics in children are limited. In two children undergoing liver transplants for end stage liver failure, one of whom had comorbid renal impairment, accumulation of morphine metabolites was only seen in the presence of renal disease (38), emphasizing the importance of kidney health in morphine clearance. Respiratory comorbidities have been identified as potential factors contributing to opioid related adverse drug reactions in children receiving codeine post-tonsillectomy (39). In cases where the respiratory tract is compromised, such as with asthma or infections the addition of an opioid can further depress the respiratory drive resulting in potentially fatal adverse effects. Children with chronic lung

disease, with impaired CO₂ response may also be at an increased risk for respiratory depression following the administration of opioids.

E) Maternal factors:

The extent of neonatal opioid exposure *in utero* and through lactation will vary according to maternal parameters including drug, dose, and duration of therapy. An increased dose and a longer duration of therapy will increase the amount of drug in maternal circulation thereby increasing the amount of drug seen by the placenta and the fetus. Furthermore any conditions that decrease maternal drug clearance will increase the amount of fetal exposure during pregnancy. Maternal polysubstance use, especially other opioids and benzodiazepines, has been shown to worsen NAS symptoms in the neonate following *in utero* methadone exposure (41-43). Buprenorphine has been reported to evoke less severe withdrawal symptoms than methadone (42). Some controversy exists surrounding the relationship between maternal methadone dose and NAS severity. Reports of an increased rate of NAS or increased NAS severity with higher maternal methadone doses (42, 44) are contrasted by those studies that do not find a dose response relationship (45, 46). Neonatal sedation resulting from codeine exposure in breast milk has been shown to worsen after 4 days of maternal exposure (40). In rodent models, exposure to soy protein isolate, potentially found in human formula, increased the expression CYP2B and CYP3A isozymes (47). When compared to breastfed infants, formula-fed infants showed an increased expression of CYP3A4 between the ages of 2-10 weeks, indicating a potential effect of diet on maturation of drug metabolism enzymes (48).

1.3 Pharmacogenetic sources of variability in pediatric response to opioids

The rapidly advancing field of pharmacogenomics seeks to explain inherited variability by assessing the contribution of an individual's genetic make-up to drug metabolism and drug response. A patient's response to codeine, morphine or methadone is highly variable, some of which can be explained by mutations in genes encoding for drug metabolism enzymes, drug transporters, target receptors and/or signalling proteins. Mutations that affect opioid response include single nucleotide polymorphisms (SNPs), insertions/deletions as well as copy number variants. This section will discuss some of the potential sources of pharmacogenetic variability and the implication for opioid response in infants and young children.

1.3.1 Cytochrome p 450 enzymes:

The cytochrome P 450 (CYP) enzymes are responsible for the phase I metabolism of roughly 80% of the drugs on today's market. These enzymes have a broad substrate specificity, oxidizing a wide range of compounds including opioids.

CYP2D6:

CYP2D6 is expressed in the liver and the CNS including the neocortex, hippocampus, substantia nigra and in Purkinje cells (49). CYP2D6 is responsible for the metabolism of ~25% of clinically used drugs, including several opioids such as tramadol and oxycodone despite its relatively low hepatic abundance. Throughout gestation, fetuses aged 14-24 weeks have

CYP2D6 protein expression ranging from undetectable to roughly 3-5% of adults values (50). Liver samples obtained from birth to postnatal day 7 had detectable CYP2D6 protein in all samples (low amounts of only 5% adult values) and expression increased to 30% adult values between 7-28 days of age and up to 70% after four weeks (50, 51). The correlation seen with adult protein expression and CYP2D6 mRNA levels does not hold true in the fetus or neonates as high mRNA expression suggests a variable age dependent translational control mechanism for hepatic CYP2D6 expression and activity.

CYP2D6 catalyzes the O-demethylation of codeine into active morphine and plays a minor role in the metabolism of methadone. Over 100 different allele variants result in considerable phenotypic variability in CYP2D6 activity (52). These phenotypes range from a poor metabolizer (PM) with little to no enzymatic activity to ultra-rapid metabolisers (UM) formed by functional gene duplication. Therapeutic failure has been reported following codeine use in CYP2D6 PM's resulting from their inability to produce the potent morphine metabolite. A qualitative measure for CYP2D6 phenotypes has been designated as the Activity Score (AS) (53). The AS is a sum of the individual allele's contribution to the rate of CYP2D6 metabolism. A PM is assigned an activity score of 0, as there is no conversion of codeine to morphine. This arises when an individual is homozygous for two non-functional alleles. The contribution of individual alleles to the overall rate of CYP2D6 metabolism is displayed in Table 1.1. A CYP2D6 UM is characterized by a functional gene duplication, and shows a gene-dose effect whereby the number of duplicated alleles increases as does the ratio of codeine converted to morphine (53, 54). The frequency of the CYP2D6 UM phenotype is ethnically diverse, and the prevalence is seen in Table 1.2. The correlation between the activity score and the ratio of

morphine to codeine is illustrated in Figure 1.4. Variability in CYP2D6 enzymatic activity creates potential for adverse effects ranging from therapeutic failure in PM's to respiratory depression in UM's due to an unpredictable increase in morphine production. The CYP2D6 UM genotype has been identified as a risk factor for CNS depression in neonates exposed to codeine in breast milk (55, 56) and for fatal respiratory depression in children receiving codeine post-adenotonsillectomy (39, 57).

In regards to the pharmacokinetics of methadone, initial reports suggested that reduced CYP2D6 activity was associated with greater therapeutic success among patients when compared to UM metabolizers (58), possibly due to a reduction in hepatic methadone breakdown. Further research has suggested that CYP2D6 plays only a minor role in methadone metabolism, and that CYP2D6 genotype does not impact methadone clearance or dosing requirements (59-61). CYP2D6 has a broad substrate specificity and although not generally inducible, can be inhibited by several selective-serotonin reuptake inhibitors such as paroxetine, sertraline and fluoxetine mimicking the effects of the PM phenotype. In patients where CYP2D6 has been inhibited, a lack of analgesic effectiveness following codeine administration has been documented (62).

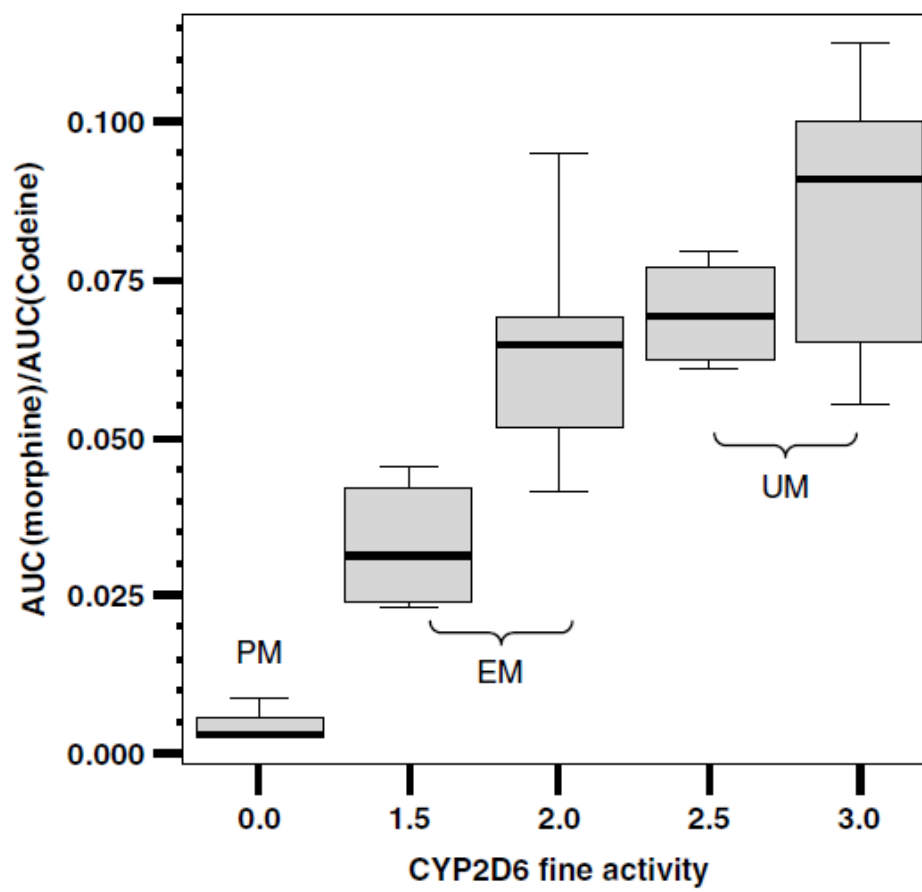
Table 1.1. The contribution of individual alleles to the rate of CYP2D6 metabolism as assessed by the activity score where N = the number of duplicated alleles (2, 53, 63)

Allele:	Activity Score (AS):	Effect on CYP2D6 activity
*1xN, *2xN	1.0 x N	Increased
*1, *2, *27, *33, *35, *45, *46, *39, *48, *53	1.0	Normal
*9, *10, *19, *17, *29, *41, *49, *50, *54, *55, *59, *69, *72	0.5	Reduced
*3-8, *11-16, *18-21, *31, *36, *38, *40, *42, *44, *47, *51, *56, *57, *62	0	No Activity

Table 1.2. Frequency of CYP2D6 ultra-rapid metabolizers (UM) in different ethnicities.

Population	UM Genotype prevalence %
Northern Africa	29% (64)
African American	3.4-6.5 % (65, 66)
South/West Africa	7.1-7.4% (67)
Western Asian	7.8% (67)
South/East Asian	1.2% (68)
South American	3.1% (67)
Caucasian	3.6-6.5% (65, 66)
Greek	6.0% (69)
Hungarian	1.9% (70)
Oceania	26%
Northern European	1-2% (71)
Western European	5.5% (71)

Figure 1.4. Ratio of plasma area-under-the-curve (AUC) of morphine to AUC of codeine correlated with the CYP2D6 fine activity (activity score) (2, 54)



CYP3A4 and CYP3A7:

CYP3A is the most abundant CYP subfamily expressed in the human liver and collectively 3A isozymes are responsible for the metabolism of 50% of drugs used clinically. CYP3A7 is the dominant hepatic isozyme in the fetal liver whereas CYP3A4 is the predominant isozyme in liver and intestine (72). CYP3A4 expression is approximately 20% of adult expression level at birth and reaches up to 60% of adult levels after one week of life (73). Hepatic expression of both fetal CYP3A7 and CYP3A4 has been correlated with the pregnane X receptor and the constitutive androstane receptor which are known to play a key role in regulating the expression of these CYP proteins (74).

CYP3A4 is responsible for converting codeine into norcodeine and is important player in the inactivation of methadone. Although most identified CYP3A4 polymorphisms do not correlate with protein expression, CYP3A4 *22 carries an intron mutation that reduces mRNA expression that has been shown to affect the pharmacokinetics of statin drugs (75). To the best of our knowledge there have been no positive correlations between CYP3A4 genotype and codeine/morphine response, however a previous case report suggested that CYP2D6 UM's are at an increased risk for morphine toxicity in the presence of the CYP3A4 inhibitors, the antibiotics clarithromycin and voriconazole (76). These inhibitors decrease the formation of the inactive norcodeine metabolite. Intestinal and hepatic CYP3A4 activities have been shown to have a minimal effect on methadone N-demethylation, explaining roughly 15% of variability in total methadone clearance (61, 77) possibly due to the compensation for low CYP3A4 activity by

other CYP alleles. Caution is also warranted for patients prescribed methadone and taking CYP3A4 inhibitors.

CYP2B6:

A great degree of variability exists in the expression and activity of hepatic CYP2B6; the relative abundance of hepatic CYP2B6 varies up to 21-fold amongst individuals. Although primarily a hepatic drug metabolism enzyme, CYP2B6 is expressed in the CNS, kidney and lungs (78). Compared to the other CYP families, CYP2B6 has a relatively small substrate specificity, metabolising only 4% of drugs used clinically (79). CYP2B6 is responsible for the metabolism of the antiretrovirals, efavirenz and nevirapine, used in the management of HIV. These antiretroviral drugs have been shown to induce CYP2B6 through activation of the pregnane X receptor and the constitutive androstane receptor via pathways similar to induction in CYP3A4 (80). In the majority of individuals, CYP2B6 is expressed at birth, throughout weeks 10-35 of gestation (81) and levels increase during the first month of life.

CYP2B6 plays a stereo selective role in metabolising S-methadone to S-EDDP (82-84). Previous work has identified a haplotype resulting from two nonsynonymous mutations (G516T and A785G) creating a poor metaboliser, or CYP2B6 *6 with roughly 50% less active protein than wild-type. Furthermore, a C1459T SNP (CYP2B6 *5) results in decreased protein expression (85). CYP2B6 *6/*6 is associated with a reduction in S-methadone metabolism (59, 86) and in methadone-related deaths, CYP2B6 *6 is associated with higher post-mortem methadone levels (87). The CYP2B6 *6/*6 genotype has been identified in 26% of Caucasians and in 16% of individuals of Japanese descent (88). CYP2B6 *6/*6 is responsible for a

significant increase in the risk for QT prolongation (25), due to a reduction in S-methadone metabolism, and is the first genetic factor that could potentially predict an increased risk for methadone related cardiac toxicity. The impact of CYP2B6 polymorphisms on neonatal withdrawal and methadone concentrations following *in utero* methadone exposure has not been investigated to our knowledge.

1.3.2 Uridine diphosphate glucuronosyltransferase

The glucuronidation pathway plays a large role in the inactivation of many xenobiotic and endogenous compounds including both codeine and morphine. Uridine diphosphate glucuronosyltransferase (UGT) 2B7 is predominately located in the liver, with reduced expression in the gastrointestinal tract, kidney, pancreas and brain (89). In a study of liver donors, children ages 1-11 showed only 25% of adult UGT2B7 activity and protein expression which reached roughly half of adult levels between the ages of 12-17 years (90). Substrates of UGT2B7 include steroid hormones, bile acids, nonsteroidal anti-inflammatory drugs and valproic acid.

UGT2B7 is a prominent phase II drug metabolism enzyme responsible for converting roughly 50-70% of codeine into codeine-6-glucuronide and producing both M3G and M6G metabolites from morphine (Figure 1.2) (91-93). Although generally a detoxication reaction to increase compound hydrophilicity, the M6G metabolite produced by UGT2B7 from morphine has an opioid receptor potency 2-4 times greater than morphine. Isolated fetal liver microsomes have only 10% of the glucuronidation activity of adult liver microsomes, a probable explanation of the reduced morphine clearance seen in the neonate (94). An amino acid transition at residue

268 from histidine to tyrosine (C802T) giving rise to the UGT2B7 *2 genotype has been shown to alter the pharmacokinetics of several of its substrates including diclofenac (95) and mycophenolic acid (96).

In one fatal case of codeine toxicity in a breastfed infant, the mother was both a *CYP2D6* UM and *UGT2B7**2/2 (1); the increased morphine formed from codeine could not be cleared by the deficient glucuronosyltransferase. There are some further studies that suggest the *UGT2B7* *2/*2 genotype can result in an increased conversion of morphine to M6G (91, 98). The combination of the *CYP2D6* UM polymorphism causing a greater amount of codeine bioactivation to morphine and the *UGT2B7* *2/*2 increased M6G production may increase the risk for CNS depression.

Theoretically, polymorphisms in UGT could affect the ratio of M3G/M6G to morphine, however the clinical impact of changes in UGT activity on morphine response is unclear. An increase in UGT activity would increase the production of both morphine metabolites and the neuroexcitatory metabolite (M3G) may attenuate the increased analgesic properties of M6G. Conversely decreased UGT activity, resulting in a decrease in M6G: morphine may decrease analgesic effectiveness. Researchers reporting on 175 cancer patients did not identify an association between M3G: morphine or M6G: morphine with ten different UGT SNPs (99). Conversely, a small cohort of sickle cell patients was shown to have reduced morphine glucuronidation in the presence of the *UGT2B7* *2/*2 genotype. These controversial findings

potentially result from inconsistent patient treatment groups, variable dosing and different routes of administration between studies, thus further investigation is warranted.

1.3.3 The P-glycoprotein transporter

P-glycoprotein (P-gp) is found on endothelial cells at the blood brain barrier, on enterocytes, as well as on biliary and renal epithelial cells. In animal models, this essential drug efflux transporter has been shown to play a role in the efflux of morphine and methadone (100-102). As the primary target of opioids is the CNS the rate of efflux from the brain can greatly influence the concentration of opioid in the brain and the resulting analgesic effects. P-gp expression in the gastrointestinal tract can influence the bioavailability of oral morphine and methadone, and hepatic and renal P-gp affect metabolism and clearance. Although a limited number of studies have sought to characterize the ontogeny of P-gp, transporter expression in the brain has been identified in fetuses aged 17-24 weeks at reduced expression (~20%) compared to adult samples (103). P-gp expression has been suggested to reach adult levels by 21 days of life (104) although this study was conducted in pigs and human studies are needed to corroborated these findings.

P-gp exhibits a 200-fold inter-individual variability in mRNA expression and up to 50 fold variability in protein expression in the liver (88). The ATP-binding cassette efflux drug transporter, P-gp is encoded by the polymorphic ABCB1 gene and over 35 coding region SNPs have been identified in this gene (63). Homozygotes for low activity ABCB1 alleles are found in roughly 25% of the Caucasian population. Of importance, when compared to the homozygous C/C wild- type, the T/T at position 3435 SNP has been associated with greater pain relief in

chronic morphine therapy (105). The loss of function in the P-gp transporter efflux system results in a greater morphine accumulation in the brain and therefore increases analgesic response (2). The C3435T has been linked to SNPs G2677T/A and C1236T (106). Other studies have challenged this dose relationship finding and found no association between the C3435T SNP and morphine dose following surgery, however combined P-gp genotype (C3435T and G2677T/A) did correlate with morphine related side effects (107). In neonates exposed to codeine in breast milk, the ABCB1 2677TT genotype in combination with CYP2D6 UM phenotype predicted neonatal CNS depression (56).

The extent of P-gp genotype effect on methadone response is unclear. Studies have shown inconsistent findings including no relationship of P-gp genotype with methadone pharmacodynamics (108). Conversely, an association between ABCB1 genotype and methadone response and plasma levels has also been described (59, 109). In P-gp knockout mice, concentrations of methadone in the CNS were found to be increased as were the analgesic effects (110, 111) suggesting an essential role of P-gp in regulating methadone response. Administration of P-gp inducers, rifampicin (112) or St Johns Wort (113), resulted in increased opioid withdrawal symptoms and decreased plasma methadone concentrations resulting from an increase in efflux from the CNS and increased clearance.

P-gp plays an essential role in efflux of opioids from placenta back into maternal circulation (114). SNPs have been shown to alter P-gp expression in the placenta (115, 116) which may have a role in determining neonatal response following *in utero* opioid exposure.

1.3.4 Organic cation 1 transporter

The organic cation transporter (OCT) 1 is an active uptake transporter present on the sinusoidal membrane of hepatocytes. Recent *in vitro* cell work supported a role of OCT 1 in the active hepatic uptake of morphine (117). Codeine and methadone have not been shown to be substrates for OCT 1 uptake. Hepatocytes overexpressing OCT 1 had a 4-fold increase in morphine uptake which was abolished in the presence of an OCT 1 inhibitor (MPP+) and by loss-of-function (LOF) polymorphisms (117). The OCT 1 protein is encoded by the solute carrier family 22 member 1 (SLC22A1) gene, for which several LOF, nonsynonymous polymorphisms (Arg61Cys, Cys88Arg, Gly401Ser, Gly465Arg) and a deletion mutation (met420del) have been identified which decrease activity (117). These polymorphisms are relatively common in the Caucasian population, with 9% homozygous and 40% heterozygous carriers of one or more of these LOF SNPS reported (117).

As the main pathway of morphine elimination is via hepatic glucuronidation, the rate of entry of morphine into the liver may have an important effect on plasma morphine levels, as well as altered pharmacodynamic effects. In a study of children undergoing tonsillectomy, OCT 1 LOF variants significantly altered the pharmacokinetics of morphine (118). Further studies are needed to confirm the findings of these early reports to confirm the role of OCT 1 polymorphisms in the pediatric response to morphine.

1.3.5 The mu-opioid receptor

The MOR is the primary binding site of opioids, and is activated endogenously by β -endorphan and enkephalin. Opioid receptors are G-protein-coupled receptors distributed throughout the central and peripheral systems including localization in the GI tract, dorsal horn, medulla and midbrain. MORs are located on the presynaptic terminals of nociceptive neurons where agonist binding results in phosphorylation of the α -subunit. Activation of this g-protein-coupled receptor through phosphorylation alters cellular levels of cyclic AMP via inhibition of adenylyl cyclase on the cell membrane (Figure 1.5). In the short term, these second messenger proteins result in the activation of protein kinases which alter the electrical excitability of ion channels affecting the regulation of numerous cellular processes (1). The increase in cAMP kinase signals potassium channels to open and sodium channels to close, resulting in hyperpolarization and presynaptic neuronal inhibition, thus preventing neurotransmitter release. This diminishes pain perception by reducing nociceptive transmission sent from the site of injury in the periphery to the thalamus for processing. In cases of chronic opioid exposure, as seen with addiction, the MOR is responsible for alterations to gene transcription. These long term changes are thought to occur in the nucleus accumbens via regulation of the cyclic AMP response element binding protein (CREB) (119, 120) (Figure 1.5).

MOR is encoded by the *OPRM1* gene for which several nonsynonomous variants have been identified (121). The A118G SNP which alters the amino acid sequence (aspartate substitution for asparagine) is the most prevalent and well characterized mu-opioid receptor SNP occurring in an extracellular glycosylation site. The A118G SNP has been reported in 10-30% of

Caucasian populations, reported more frequently in Asians (48%) and less frequently in African Americans (5%) (122-124). Altered receptor binding characteristics (125) and changes in mRNA expression (122) have been proposed as potential mechanisms behind the A118G altered opioid response although the literature is contradictory.

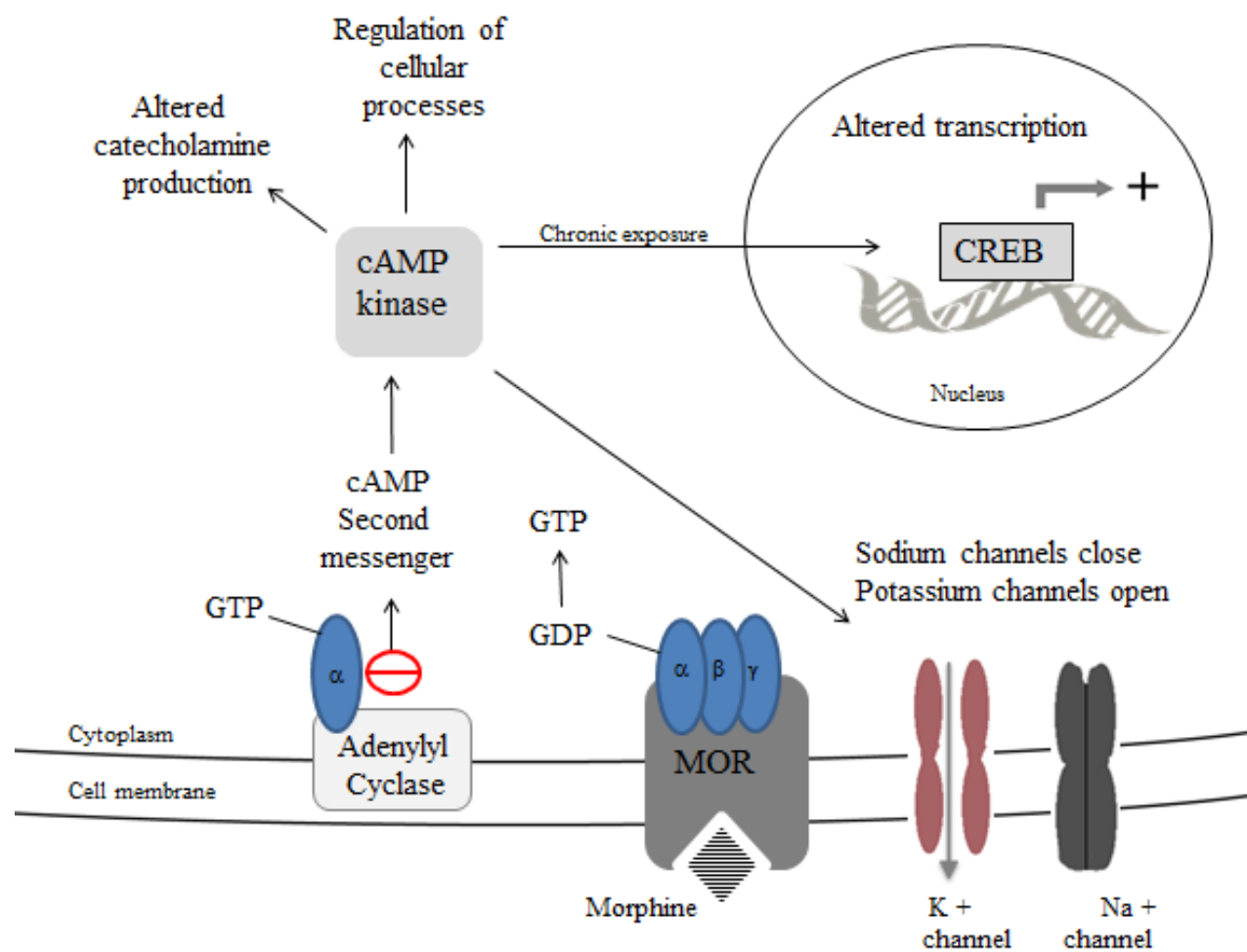
In experimental pain models, homozygous carriers of the A118G SNP were shown to require 2 to 4-times as much alfentanil as the wild-type carriers (126). Ten-fold higher concentrations of drug were required to produce the same amount of respiratory depression, suggesting a protective role of the homozygous mutation on respiratory effects of opioids. Investigations in three cohorts of patients receiving post-surgical morphine for abdominal hysterectomy (127), total knee arthroscopy (128) and major abdominal surgery (129) identified significant increases in morphine requirements in homozygous GG carriers within 24 hours of surgery. Furthermore, cancer patients carrying at least one mutated OPRM1 A118G allele were less likely to respond to morphine, reporting significantly higher pain scores (105). These studies are not without their limitations, as they report on different morphine doses, treatment durations, often do not account concomitant medications and disease conditions, but do overall suggest an increased opioid requirement in adult carriers of the A118G SNP. There are limited studies assessing the effects of OPRM1 A118G on morphine response in children the results of which are inconclusive.

The analgesic effects of methadone are also elicited through activation of MOR signalling pathways. When assessing the central nervous system effects of R-methadone via

miosis, carriers of at least one A118G mutation had a lower percent change in pupil size, significantly lower than wild-type individuals (108). In a study of individuals on methadone maintenance therapy, OPRM1 alone was not associated with methadone maintenance dosing (130), however in a polygenic regression model (including ABCB1 and CYP2B6) was able to explain 53% of the variability in methadone dosing requirements (130).

Several other polymorphisms have been described with a minor allele frequency >1%. A serine to proline substitution (C802T) results in altered signalling and receptor desensitization by reducing G-protein coupling and is associated with decreased morphine potency (131). Further, SNPs G799A and G749A both which encode intracellular arginine to histidine transitions are associated with reduced signalling, however the effects on opioid potency have not been established (132). Although the cellular mechanism is unclear and the effects of the OPRM1 A118G on pediatric opioid response are not well characterized, the current literature suggests further investigation of OPRM1 in genetically determined variability in pediatric opioid response are required.

Figure 1.5. Mechanism of action of opioid at the mu-opioid receptor (120, 133)



1.3.6 Catechol O-methyltransferase

Catechol-O-methyltransferase (COMT), an enzyme responsible for the metabolism of catecholamines, is a key modulator in the neurotransmissions associated with the perception of pain. COMT is responsible for the inactivation of dopamine, epinephrine and norepinephrine and COMT regulation of dopaminergic and adrenergic transmission has important implications for the mu-opioid response system (134). A substitution of methionine for valine at codon 158 has been associated with a 4-fold reduction in COMT enzyme activity. COMT Val158Met SNP has been shown to affect the binding capacity of the MOR via altering the density of receptors (135) (136). In a study by Rakvag et al, cancer patients with the COMT val/val genotype required much more morphine than the met/met genotype ($155 \pm 160\text{mg}$ vs. 95 ± 99 mg, respectively)(136). In this cohort the met/met genotype was identified in 32% (67/207) of Caucasian patients. Further work by this group established the combined effect of COMT and OPRM1 where carriers of the met/met and 118 AA (OPRM1 wild-type) genotype needed significantly less morphine to effectively treat cancer pain (135). The role of COMT in modulating dopaminergic activity may affect the density of opioid receptors as the met/met genotype has been associated with an increase in MOR binding potential (134). Along with polymorphisms in OPRM1, infants with the A158G COMT variant had a significantly shorter length of stay in hospital, and were less likely to require pharmacological management following *in utero* opioid exposure (137). Reduced COMT activity and subsequent reduction in dopamine breakdown may play a protective role in the development and severity of neonatal abstinence syndrome, however further studies should be conducted in order to corroborate the findings of this initial study.

1.4 Thesis objectives and hypothesis:

We hypothesize that clinical and genetic factors will create variability in the response of young children to opioids.

This study investigates clinical and genetic risk factors for opioid toxicity in three pediatric populations; firstly, neonates exposed to methadone *in utero*. Secondly, I examined the safety of infants exposed to codeine through breast milk following labour, and finally assessed the response of young children to opioids used for post-tonsillectomy pain management. The objective of my thesis is to identify potential predictors of opioid morbidity and mortality in young children by assessing risk factors.

Specific Research Objectives:

Objective 1: To investigate risk factors for neonatal abstinence syndrome following *in utero* methadone exposure

Objective 2: To assess the use of clinical guidelines in reducing neonatal sedation following codeine exposure through breast milk

Objective 3: To evaluate post-operative opioid use in children with obstructive sleep apnea:

- a. Identify clinical and genetic factors that contribute to fatalities in young children following codeine use post-tonsillectomy for OSA
- b. Investigating the safety and effectiveness of morphine and ibuprofen in managing pediatric pain post-tonsillectomy

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Chapter 2: In utero opioid exposure and neonatal abstinence syndrome

Part of this chapter has been adapted from published work:

Kelly LE, Rieder M, Brigdman-Acker K, Lauwers B, Madadi P, Koren G. Are infants exposed to methadone *in utero* at an increased risk for mortality? *J Popul Ther Clin Pharmacology* 2012; 19 (2) e160-166

2.1 Introduction: Opioid use and abuse in pregnancy

2.1.1 Prevalence

The prevalence of opioid dependence amongst women has been on the rise in many North American jurisdictions over the last 5 years (1). It has been estimated that approximately 90% of drug-abusing women are within childbearing age, and the National Survey of Drug Use and Health revealed that 4.4%-16.2% of pregnant women reported using illicit drugs (2-4). Furthermore, opioid abuse stigmatization and concerns surrounding loss of custody often results in an underestimation of the true prevalence of opioid use during pregnancy (4). The College of Physicians and Surgeons of Ontario (CPSO) has estimated that oxycodone prescriptions have increased by approximately 850% from 1991-2007 which parallels the rise in Ontarians requiring methadone maintenance therapy (MMT), from roughly 700 to 16,400 over the same time period (1-3, 5). During pregnancy both illicit and prescription opioid use can result in a variety of gynecological and obstetrical complications.

2.1.2 Management of maternal addiction with methadone

MMT has been practiced in North America as the preferred method of treating drug dependency since the 1960s, and remains the current standard of care (5). MMT is generally considered safe and is recommended for opioid-dependent women during pregnancy by the American Academy of Pediatrics and the Centre for Substance Abuse Treatment (2). The benefits of MMT include improved birth weight, decrease in infant mortality, decreased withdrawal symptoms in both mother and baby, and a decrease in the dangers associated with maternal drug seeking behaviours (6) While crossing the placenta, methadone has not been identified as a human teratogen (5). Infants exposed to methadone *in utero* commonly display signs of opiate withdrawal after birth in up to 85% of cases (7). Currently, information

regarding the risk of mortality and the long-term effects of *in utero* methadone exposure is scarce (8). Alternatives to methadone include buprenorphine, or the combination of buprenorphine and naloxone (Suboxone ®) and require further long-term research before recommendations for use in pregnancy can be made.

2.1.3 Neonatal Abstinence Syndrome

Opioids, including methadone and heroin have been shown to cross the placenta (9) (10) and *in utero* exposure can lead to neonatal withdrawal. In Ontario, the incidence of neonatal abstinence syndrome (NAS) diagnosis has closely paralleled the increase in rates of known maternal addiction. This increase has been associated with a significant burden on neonatal intensive care units across the province. Approximately 85% of babies exposed to methadone *in utero* develop at least one sign of NAS, however this number has been reported to range between 13-94% (6, 11, 12). In 2010, the incidence of NAS in Ontario was estimated at 4.3 cases per 1000 births (4).

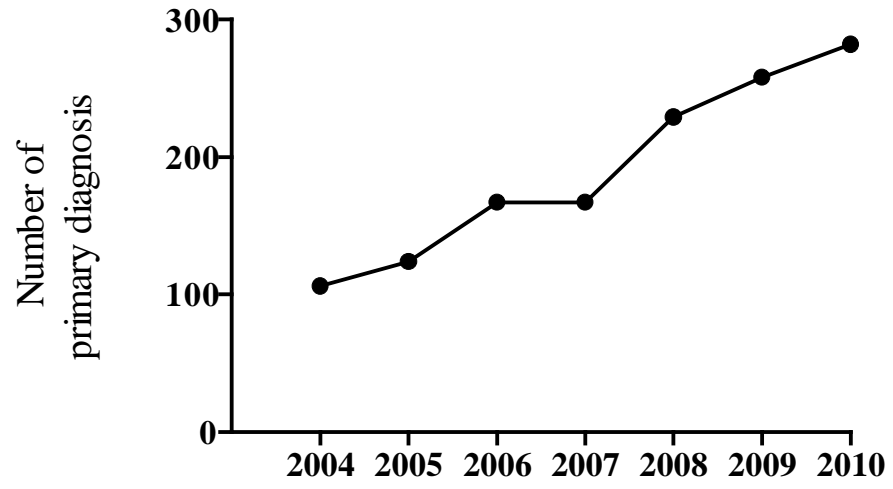
The symptoms of NAS include central nervous system (CNS) hyperirritability, seizures, poor feeding, and metabolic and respiratory disturbances (11). With the increase in the number of infants exhibiting signs of NAS (13), there has been a clinical impression that there may be a higher rate of mortality amongst this group of young methadone exposed infants, than that of the general population. While non-pharmacologic management, including swaddling, low lighting, breastfeeding and minimal contact can often mitigate NAS symptoms roughly 60% of neonates exhibiting severe symptoms and will require pharmacologic intervention (14) . Weaning doses of morphine are the more frequent

pharmacologic intervention, with concurrent phenobarbital or clonidine for infants who's NAS is not well controlled with morphine (4, 15). Costs associated with US public Health for neonates who were exposed to *in utero* opioids was estimated to be up to \$720 million USD for 2009 alone (16). The NAS severity, and the time of onset of withdrawal symptoms are complicated to determine and unpredictable (17). Chapter 5 contains further information regarding NAS treatment with oral morphine and weaning protocols in London, Ontario.

Breastfeeding has been shown to reduce the severity of NAS by providing small amounts of methadone through milk (18, 19). Prematurity has been associated with a reduced NAS severity due to an immature CNS, less accumulation of drug in fatty tissue and an overall decrease in length of exposure (7, 20). Further clinical risk factors for NAS potentially include gender, in which one study reported male babies with a greater need for pharmacologic intervention (21), maternal polysubstance use (20, 22, 23), and duration of exposure. Controversy exists regarding the association between maternal methadone dose and NAS severity (7, 22).

The impact of genetic polymorphisms on the development and severity of NAS following methadone exposure has only been reported by one group (24). Watchman et al. investigated the impact of SNPs in OPRM1, COMT and ABCB1 with the length of stay and the need for treatment of NAS. Variants in OPRM1 and COMT genes showed a protective effect and were associated with a decrease in hospital stay and less need for pharmacologic treatment. This group did not investigate the effects of polymorphisms in drug metabolism enzymes on methadone levels in the neonate or on overall NAS progression.

Figure 2.1. Prevalence of primary NAS diagnosis in the province of Ontario



2.2 Are neonates exposed to methadone *in utero* at an increased risk for mortality?

The objective of this investigation was to quantify the rates of infant mortality amongst in utero methadone-exposed children younger than one year of age and to compare it to that of the general population in Ontario, Canada.

2.2.1 Methods

Several provincial and national databases were employed to retrieve the required data for this investigation:

1) Information was obtained on all cases of child fatalities in Ontario identified as exposed to methadone *in utero* from January 2006 to December 2010. These data are recorded at the Office of the Chief Coroner of Ontario (OCC). A paediatric death investigation was completed for all cases in accordance with the OCC's policy, which included the Protocol for the Investigation of Sudden and Unexpected Deaths in Children Under Five Years of Age. The information collected included; demographics, drugs of exposure, post-mortem reports, toxicology screens, and diagnosed causes of death.

2) The number of cases of NAS diagnosis in Ontario was obtained from the Canadian Institute for Health Information (25). CIHI data are collected in accordance with the Standards for Management Information Systems in Canadian Health Service Organizations (MIS standards) and were reported by the Provincial Council for Maternal and Child Health(13, 25). MIS

standards are a set of national guidelines for gathering and processing data, reporting financial and statistical data on the day-to-day operations of a health service organization. They also provide a framework for integrating clinical, financial and statistical data when recipient costing is done(25).

3) The Ontario Infant Mortality Rate (IMR) report which includes data on deaths of children up to one year of age was obtained from Statistics Canada(26). This value is presented as rates per 1000 live births in a given year. The IMR used for the present study was the latest available, from 2007(26), and it follows the International Statistical Classification of Diseases and Related Health Problems (27) Revision No.10.(28) The published reference values for age-adjusted normal organ weights were obtained from Coppoletta & Wolbach, which is based on analysis of 2287 autopsy records at children's and infants' hospitals in the United States(29). Only those organs with no demonstrated pathological changes and no diseases or abnormalities noted were included in our analysis. Confidence intervals using regression equations for the original Coppoletta & Wolbach data were later published by Shankle et al in 1983 (30). Reference values were compared with the organ weights obtained from the fatal cases associated with *in utero* methadone exposure in Ontario.

Statistical analysis was performed by determining an odds ratio (OR) and 95% confidence interval was calculated to compare the mortality risk of infants <1 year of age exposed in utero to methadone and experiencing NAS to the risk in the general population of Ontario. Reference weights were compared to methadone cases using age at the time of death (months) using a Mann-Whitney U Test.

2.2.2 Results

Between January 1, 2006 and December 31, 2010, the Office of the Coroner of Ontario identified 8 deaths of children younger than one year whose drug dependent mothers were on MMT. Upon investigation by the Office of the Chief Coroner and a Pediatric Death Under Five investigation, six of the eight deaths were classified as Sudden Unexpected Death Syndrome (SUDS), all of whom had evidence of unsafe sleeping environments, three of which also included bed sharing. One fatality was determined to be hypoxic ischemia encephalopathy due to bathtub drowning, and the cause of one death was unascertained. Comparison of organ weights at the time of autopsy with published age-specific reference organ weights showed a significant increase in both right and left lung weight ($p = 0.035$, $p = 0.007$ respectively) (Table 2.1). A non-significant trend towards increased liver, brain and heart weights compared to reference values was demonstrated. There were four female and four male children with a median age at the time of death of 6 months (range 1.4-11). No deaths were reported in the neonatal period (first 28 days). The median maternal age was 27 years (range 23-32) with a median gestational age at birth of 35 weeks (range 32-39). Only three mothers (37.5%) reported initiating breastfeeding. Seven of the eight (87.5%) infants were monitored by the Children's Aid Society. In this cohort, 38% (3/8) of mothers reported concomitant use of oxycodone and 50% (4/8) reported cocaine use during pregnancy. Post-mortem blood toxicology tests using gas chromatography and mass spectrometry did not detect morphine, methadone, alcohol, cannabinoids, or cocaine in any of the eight cases. In one case opiates, cocaine and cocaine metabolites were detected in the child's hair. In another case therapeutic levels of acetaminophen and pseudoephedrine ($<50\text{mg/L}$) were identified in the blood of an infant who had been given common cold medications.

Table 2.1. Organ weights in comparison to normalized age-adjusted reference weights compared using a Mann-Whitney U test for significance.

CASE:	Right Lung (g)	RL REF	Left Lung (g)	LL REF	Heart (g)	HR REF	Liver (g)	LV REF	Brain (g)	BR	Right Kidney (g)	RK REF	Left Kidney (g)	LK REF
#1	128	55	105	50	42	40	225	225	895	875	23	32	25	33
#2	36	45	N/A	N/A	40.5	30	223	175	740	630	23.6	26	22.3	25
#3	50.9	32	40.2	29	28.3	24	198.3	140	509.9	425	19.2	20	19.5	20
#4	97	55	70	50	41	40	324	225	1001	875	25	32	34	33
#5	49.85	45	49.85	40	30.8	30	240	175	760	630	18.8	26	18.8	25
#6	56.5	32	51	29	21.5	24	176.5	140	442	425	23	20	25	20
#7	81.8	47	70.4	43	43	33	360	200	950	675	28.1	27	31.2	23
#8	59	32	66	29	25.6	24	148.3	140	557	425	15.8	20	19.7	20
p value =	0.035		0.007		0.368		0.054		0.252		0.310		0.788	

There were no other toxicologically-relevant compounds identified by immunoassay or gas chromatography- mass spectrometry. Based on CIHI records there were 821 recorded NAS diagnoses in Ontario from 2006-2009. We extrapolated these data by linear regression and estimated approximately 282 NAS diagnoses in 2010 (Figure 2.1). The mortality rate of infants exposed to in utero methadone was therefore 8 per 1103 children diagnosed, or 0.725%.

According to Statistics Canada, the Infant Mortality Rate (IMR) in the Province of Ontario for the same time period was 5.2 per every 1000 live births up to one year of age (Statistics Canada, 2011). This yields an odds ratio of 1.45 for mortality in children with NAS (95% confidence interval 0.47-4.46) ($p = 0.56$).

2.2.3 Discussion

In evaluating the causes of infant death amongst the eight infants exhibiting NAS at birth, several etiological directions have to be considered. Firstly, only three of eight mothers admitted initiating breastfeeding, as a potential source of continuous methadone exposure. The other 5 mothers did not provide data for this point. The toxicology screens did not detect any methadone or drugs of abuse in post-mortem blood samples. However, the limit of detection for these samples may not have detected low levels. The positive hair test for cocaine identified in one case possibly reflects passive exposure to cocaine smoke in the household, which has not been associated with infant mortality. In all six cases of Sudden Unexpected Death Syndrome, there was evidence of unsafe sleeping environment and three of which included bed sharing. While bed sharing is the social norm in many cultures, its safety

and benefits have been the subject of much controversy. Bed sharing has been associated with an increased likelihood of breastfeeding frequency and duration and may strengthen the bonding between the mother and child (31, 32) The dangers of unsafe sleeping environment include an increased risk for accidental asphyxia (33).

Our study does not detect an apparent increased risk of mortality among infants born with NAS in Ontario. MMT has been recommended for use during pregnancy and has numerous maternal and infant benefits. The addict lifestyle can compromise the ability of a pregnant woman to live in a safe, substance-free environment, receive appropriate prenatal care, and maintain a healthy well-balanced diet. MMT encourages prenatal care, and is associated with an increase in birth weight and a decrease in infant mortality when compared to those addicts who remain untreated (34).

The increase in lung weights in all 8 fatal cases compared to reference values is likely due to pulmonary congestion and edema. An increase in post-mortem lung weight is consistent with previously established pulmonary pathology associated with methadone related deaths (35). The trend toward an increase in liver and brain weights in babies exposed to methadone could potentially be explained by hemodynamic perfusion alterations. Increased brain weights have been previously associated with the Sudden Infant Death Syndrome although the mechanism has not fully been described (36).

There are several limitations to the data used by us which must be considered when examining our analysis. First, CIHI information depends on appropriate recognition of NAS as well as accurate hospital coding. The Finnegan Scale, which is most commonly used to identify and quantify NAS is a partially- subjective, observation- based scale (12). Although validated; there still exists the potential that some cases of NAS may go undiagnosed. Furthermore, NAS is commonly diagnosed by confirmation of in utero opioid exposure through physical and neurological examination, maternal sampling or reporting. This may leave a group of infants exposed to methadone unaccounted for. It is therefore conceivable that there have been more cases of NAS not captured by CIHI as a primary diagnosis. An increase in this denominator would decrease the estimate of mortality odds ratio towards unity. Another limitation is the proper identification of neonates and infants who die in the first year of life who were exposed to methadone in utero and had the Neonatal Abstinence Syndrome. Unless this information were proffered by the parents at the time of death, or the investigating coroners thought to make the inquiry, the Protocol for the Investigation of Sudden and Unexpected Deaths in Children Under Five Years of Age used in these years did not make a specific inquiry into in utero exposure to methadone or NAS. Recently the OCC has undertaken a revision of the death questionnaire for infants who die in the first year of life, which will specifically inquire into maternal methadone use and the Neonatal Abstinence Syndrome, which will improve data collection. Out of the eight fatalities described here, three of the mothers also reported concomitant oxycodone use and four reported cocaine use during pregnancy. In our study, 67% (4/6) of infant mortalities where ethnicity was known were identified as First Nations. Compared to the rest of Canada (non-aboriginals) the infant mortality rate has been 1.5-4 times higher in First Nations communities (37). This

confounding factor further decreases the relative odds ratio for increased infant mortality associated with in utero methadone exposure.

In summary, maternal addiction to opioids does not appear to increase the risk of mortality among infants younger than one year of age in Ontario. While more studies are needed to corroborate these findings in other jurisdictions, it is possible that the calculated non-significant odds ratio of 1.45 is in fact even lower, due to obvious underreporting of maternal addiction during pregnancy.

2.3 Pharmacogenomics of infant deaths associated with methadone exposure

Genotyping was completed on the eight fatalities previously described. Infant DNA was purified from post-mortem blood samples using the QIAmp DNA purification system (Qiagen, Toronto, ON, Canada) according to the manufacturer's protocol. DNA samples were genotyped for variants in *CYP2B6*, such as *CYP2B6**6 characterized by variants 516G>T (rs3745274), 785A>G (rs2279343), and *CYP2B6* *2 and *10 identified by 5071 C>T (rs8192709) and variants in *CYP3A4* (rs41303343, rs55965422, rs5579860, rs55785340, rs800667, rs17342647) using a custom Illumina GoldenGate genotyping assay (Illumina, San Diego, CA, USA) according to manufacturer's protocol. DNA samples were also genotyped for genetic variations in the *ABCB1* gene: 61 A>G (rs9282564), 1236C>T (rs1128503), 2677G>T/A (rs2032582), and 3435C>T (rs1045642), *COMT* (rs165815, rs4633, rs4818, rs740602) and *OPRM1* gene 118 A>G (rs1799971) using TaqMan® genotyping assays (Applied Biosystems, Foster City, CA, USA), as previously described (Sistonen et al, Clin Phar Ther, 2012). Global minor allele frequencies were obtained from the National Center for

Biotechnology Information (NCBI) Short Genome Database available at <http://www.ncbi.nlm.nih.gov/projects/SNP/> . The sample contains a default global population of 1094 individuals across the world. As the children in this sample are of mixed decent this sample was chosen as a comparative baseline allelic distribution. More information on how this sample was obtained and analyzed is available through the Human Genome Project at <http://www.1000genomes.org/node/506>.

Table 2.2 displays the genotype prevalence in these fatalities associated with *in utero* methadone exposure as well as the minor allele frequencies present in the global population. While it appears that SNPs in P-gp may occur more frequently in fatalities following *in utero* methadone exposure, it is important to note that methadone levels were undetectable in all samples. Furthermore all eight deaths occurred in the post- neonatal period, ranging from 1.4 to 11 months of age, when all methadone exposure from maternal circulation would be cleared. It is possible that carriers of these polymorphisms had diminished methadone efflux through the placenta, the effects of which should be investigated further.

Table 2.2. Genotype of fatalities following in utero methadone exposure compared to the global minor allele frequency Data is presented as median (range) for all demographic variables. All statistical analysis comparing infant fatality and global minor allele frequencies was performed using a Fisher Exact test.

GENE:	ABCB1	ABCB1	ABCB1	ABCB1	COMT
SNP:	1236 C>T	61 A>G	2677G>T/A	3435 T>A/C	408 C>G
# homozygous:	2	0	2	0	1
# heterozygous:	2	3	4	5	1
Total # of carriers:	4	3	6	5	2
Allele count (minor)	6	3	8	5	3
CASES MAF =	0.43	0.21	0.57	0.36	0.21
Global MAF ^a =	0.42	0.04	0.34	0.40	0.34
p value	0.21	0.01	0.04	0.21	0.15
GENE:	CYP2B6 *4	CYP2B6 *9	CYP3A4	OPRM1	
SNP:	785A>G	516G>T	1047 C>T	118 A>G	
# homozygous:	1	1	0	0	
# heterozygous:	2	2	2	2	
Total # of carriers:	3	3	2	2	
Allele count (minor)	4	4	2	2	
CASES MAF =	0.29	0.29	0.14	0.14	
Global MAF ^a =	0.26	0.27	0.05	0.19	
p value	0.23	0.23	0.11	0.26	

2.4 Pharmacogenomic predictors of neonatal abstinence syndrome and enantiomeric methadone levels in the newborn

2.4.1 Introduction:

Illicit drug use during pregnancy is estimated at 16.2% in teens, and up to 7.4% amongst those aged 25 and younger (38). Methadone maintenance therapy (MMT) is the preferred method of treating drug dependency in pregnancy (5). The benefits of MMT include an increase in birth weight, decrease in infant mortality, decreased withdrawal symptoms, improved access to prenatal care and a decrease in the dangers associated with maternal drug seeking behaviours (5, 38).

Methadone is a synthetic opioid commonly administered as a racemic mixture (39, 40). Methadone is primarily metabolized in the liver; by conversion to inactive metabolites via cytochrome p450 (41) 2B6 and CYP3A4 with a minor role for CYP2D6 and CYP2C19 (40). CYP2B6 is highly polymorphic with a poor metabolizer genotype (*6) resulting from two non-synonymous mutations A785G (*9) and G516T (*4). These mutations result in a change of two amino acid residues resulting in aberrant splicing, reduced mRNA expression and subsequently decreased functional metabolic capacity (42). Previous reports have associated the *CYP2B6**6 genotype with higher trough methadone concentration and increased post-mortem methadone blood levels compared to wild-type (40, 43, 44). Concrete associations between methadone levels and polymorphisms in CYP2D6 have not yet been established (45).

It has been demonstrated that the P-glycoprotein (46) efflux transporter regulates the transfer of methadone across the human placenta (47). P-gp is further expressed in the liver, gastrointestinal tract, and at the blood-brain barrier (45) and plays an essential role in transporting methadone out of the brain and the placenta. There have been several polymorphisms associated with increased central nervous system effects of opioids by decreasing efflux out of the brain (48).

The mu-opioid receptor is the preferential binding site of methadone. Encoded by the polymorphic *OPRM1* gene, variability in this receptor has been linked to susceptibility of developing opioid addiction, increased opioid requirements and reductions in β -endorphin binding (49-51). A single nucleotide polymorphism (SNP) at 118 A>G results in an amino acid change from asparagine to aspartic acid has been shown to affect the efficacy of opioids like methadone (51, 52). The wild-type (A118) has been shown to have approximately ten times more binding sites than the G118 potentially accounting for a decrease in methadone response seen in homozygous carriers of this SNP (52). Through the mu-opioid receptor, levels of circulating neurotransmitters (dopamine, serotonin) are altered in the drug-reward pathway. Catechol-o-methyl transferase is responsible for the breakdown of catechol neurotransmitters and polymorphisms in this enzyme have been associated with an increased risk for opioid addiction (53).

The impact of genetic polymorphisms on the development and severity of NAS following methadone exposure has only been reported by one group (24). Watchman et al. explored the impact of SNPs in *OPRM1*, *COMT* and *ABCB1* with the length of stay and the need for

treatment of NAS. Variants in OPRM1 and COMT genes showed a protective effect and were associated with a decrease in hospital stay and less need for pharmacologic treatment. The effects of polymorphisms in drug metabolism enzymes on methadone levels in the neonate and on overall NAS progression have yet to be investigated.

The overall objective of this prospective pilot study is to determine the feasibility of recruiting pregnant women taking methadone and to establish the logistics of collecting neonatal and cord blood samples at the 2 participating hospitals. Auxiliary objectives include investigating pharmacogenomic predictors of NAS severity and enantiomeric analysis of placental (cord blood) and neonatal levels following *in utero* methadone exposure.

2.4.2 Patients and methods:

Ethics approval was received from the institutional research ethics board (REB) at the University of Western Ontario. Women were recruited from the local methadone clinics through their health care team, which included a pharmacist and family physician. Consenting women were recruited prior to delivery. A sample size of 20 was chosen due to feasibility and convenience sampling for this pilot study. At delivery placenta and cord blood samples were obtained by the obstetrical nurse. At 24hrs of age a 500µl sample was collected in conjunction with routine blood work for the Ontario Newborn Screening program. Neonates remained in Hospital until deemed ready for discharge. In hospital neonates were monitored for symptoms of NAS using a standardized Finnegan Scale. Neonates, who had consecutive scores above 8, were treated with oral morphine as per the institutional NAS treatment protocol. If morphine treatment was initiated weaning was at the discretion of the clinical staff. All decisions

regarding hospital discharge and at home-oral morphine weaning were made by the clinical team and were not influenced by the research staff. All parents were trained on the signs and symptoms of NAS and invited to return to hospital should these symptoms appear in their neonates following discharge. Telephone interviews were planned for 1 and 2 weeks following hospital discharge to assess any remaining withdrawal symptoms, or health concerns regarding mother and baby.

Genotyping:

Genomic DNA was isolated from infant blood samples with the Gentra Puregene extraction kit (Qiagen, Alameda, CA, USA) according to the manufacturer's protocol. DNA samples were genotyped for CYP2B6 516G>T (rs3745274), CYP2B6 1459 C>T (rs3211371), ABCB1 1236C>T (rs1128503), ABCB1 2677G>T/A (rs2032582), ABCB1 3435C>T (rs1045642), COMT 472G>A, and OPRM1 118 A>G (rs1799971) using TaqMan® genotyping assays (C__7817765_60, C__30634242_40, C__7586662_10, C_11711720C_30, C_11711720D_40, C__7586657_20, C__25746809_50, C_8950074_1; Applied Biosystems, Carlsbad, CA, USA) on a 7500 Real-time PCR system (Applied Biosystems). CYP2B6 785A>G (rs2279343) genotype was determined by sequencing.

Measuring drug levels:

Methadone was extracted from serum and placenta samples using a solid-phase C18 Speed-disk SPE cartridge pretreated with methanol and water prior to use. A sample of 200µl was loaded and washed with 0.5ml water and 200ml methanol/water (50/50). Methadone and EDDP were eluted into test tubes with 0.5ml methanol modified with TEA/FA (0.01% each). Following evaporation at 40°C and residual reconstitution in 50µl mobile phase, 20µl was

injected onto the analytical AGP chiral column. This assay was performed using an Agilent LC-Thermo MS system using a mobile phase consisting of 14% ACN: 86%NH₄ formate. The column was maintained at room temperature. Data was acquired resulting in a total ion current (TIC) plot with values of 278 for methadone and 310 for EDDP. Retention times are 6.0mins for R-methadone, 7.6mins for S-methadone, 4.2mins for R-EDDP and 5.0mins for S-EDDP. All quantification was performed manually using external standard quantitation (ETSD) methodology.

2.4.3 Results:

Between May 8, 2009 and August 11, 2010 26 pregnant women using methadone were approached to participate in this study, of which 22 provided informed consent and delivered healthy babies. Mothers were on average 26.55 ± 6 years of age and the majority (81%, 13/16) received prenatal care (Table 2.3). All neonates recorded Apgar scores of 9 at 5 minutes and there were no resuscitation attempts required at delivery. There was one premature birth (36/4 weeks) and two low birth weight babes (2390g, 2410g) in this cohort. The mean length of stay in hospital (LOS) was 8.94 ± 9.11 days. Maternal methadone dose at delivery was a mean dose of 70 ± 31 mg per day. All mothers reported addiction to opioids (oxycontin, heroin, and oxycodone) as the reason for their maintenance on methadone. Methadone use throughout the entire pregnancy was reported by the majority of mothers (67%) and the shortest duration of *in utero* exposure was 3 months. Maternal methadone dose at delivery did not correlate with placental or neonatal methadone levels obtained at 24hours of life (Figure 2.2).

While all neonates exhibited some symptoms of NAS only 36% (8/22) required pharmacologic intervention. Clinical characteristics of those who required pharmacologic intervention can be found in Table 2.4. All pharmacologic management was done by administration of morphine, except in one case where phenobarbital was used. Breastfeeding was significantly associated with less need for pharmacologic intervention, and a shorter LOS ($p > 0.01$). Neonates who were managed without pharmacologic intervention were significantly smaller, despite being of the same average gestational age. Further meta-analysis of breastfeeding and birth weight revealed a significant effect of breastfeeding in reducing the need for pharmacologic management ($p = 0.03$) and no effect of birth weight ($p = 0.98$). Previously identified clinical risk factors including polysubstance use, smoking, duration of exposure and maternal methadone dose were not associated with the need for pharmacologic management of NAS in this cohort. The effects of prematurity could not be assessed in this pilot as only one babe was born prior to 37 weeks. Genotype frequencies and NAS severity measures (LOS, need for pharmacologic treatment) are shown in Table 2.5. The rate of methadone (S)-enantiomer breakdown (S-EDDP/S-methadone) is displayed according to CYP2B6 genotype in Figure 2.3. The mean placental methadone level was 176.67 ± 119.03 ng/ml (R-methadone) and 113.17 ± 80.04 ng/ml (S-methadone). The mean placental concentration of R-EDDP was 14.39 ± 9.75 ng/ml and 24.25 ± 21.62 ng/ml for S-EDDP. In the neonate methadone concentrations measured an average of 73.20 ± 71.32 ng/ml (R-methadone) and 39.64 ± 37.29 (S-methadone). The methadone metabolites measured in neonates had average concentration of 3.56 ± 5.96 ng/ml and 5.21 ± 9.05 ng/ml for R-EDDP and S-EDDP respectively. Follow-up was not completed with any participants.

Table 2.3. Maternal and infant demographics presented as mean and standard deviation or percent.

Maternal and Neonatal Demographics:	Mean \pm SD or % (No.)
Neonates (N = 22):	
Male	29% (6/21)
Breastfed	67% (10/15)
Mean Gestational Age (54)	39.34 \pm 1.23
Mean Birth Weight (grams)	3180 \pm 525
Mothers (N = 22):	
Age (years)	26.82 \pm 5.68
Parity	0.85 \pm 1.72
Methadone duration during pregnancy (months)	7.81 \pm 2.02
Hep C Positive	5% (1/22)
HIV Positive	5% (1/22)
Smoking (yes)	94% (15/16)
Polysubstance Use	54% (7/13)
Reason for methadone use:	
Oxycontin	57% (8/14)
Oxycodone	29% (4/14)
Illicit substance (cocaine, heroin)	14% (2/14)
Concomitant substances:	
Antidepressants	23% (3/13)
Benzodiazepines	23% (3/13)
Marijuana	23% (3/13)
Psychostimulants	8% (1/13)

Table 2.4. Clinical characteristics of neonates who required pharmacologic intervention to manage their NAS and those managed with non-pharmacologic techniques. Data is presented as mean \pm standard deviation. Statistical analysis was performed using an unpaired students t-test for continuous variables, and Fischer's exact test for dichotomous variables.

	Pharmacologic intervention (N=8)	Non- pharmacologic management (N = 14)	P value
Maternal Age	26.63 ± 5.76	26.50 ± 6.24	0.96
Prenatal Care	83% (5/6)	80% (8/10)	1.00
Birth weight percentage for GA	72 ± 27	40 ± 32	0.04
LOS (days)	20 ± 11.48	3.54 ± 1.81	0.0001
Highest NAS score	13.75 ± 2.28	6.08 ± 2.20	0.0001
Smoker	88% (7/8)	100% (8/8)	1.00
#cigs/day	13.63 ± 10.20	10.63 ± 7.25	0.50
Breastfeeding	33% (2/6)	89% (8/9)	0.04
Methadone dose (mg/day)	81.75 ± 29.45	61.18 ± 29.31	0.15
Duration of exposure	8.07 ± 1.64	7.62 ± 2.20	0.67
Polysubstance use (yes)	50% (3/6)	63% (5/8)	1.00

Figure 2.2. Total methadone measured in the placenta and in the neonate correlated with maternal methadone dose at the time of delivery (mg/day). R^2 neonate = 0.01189, R^2 placenta 0.02072.

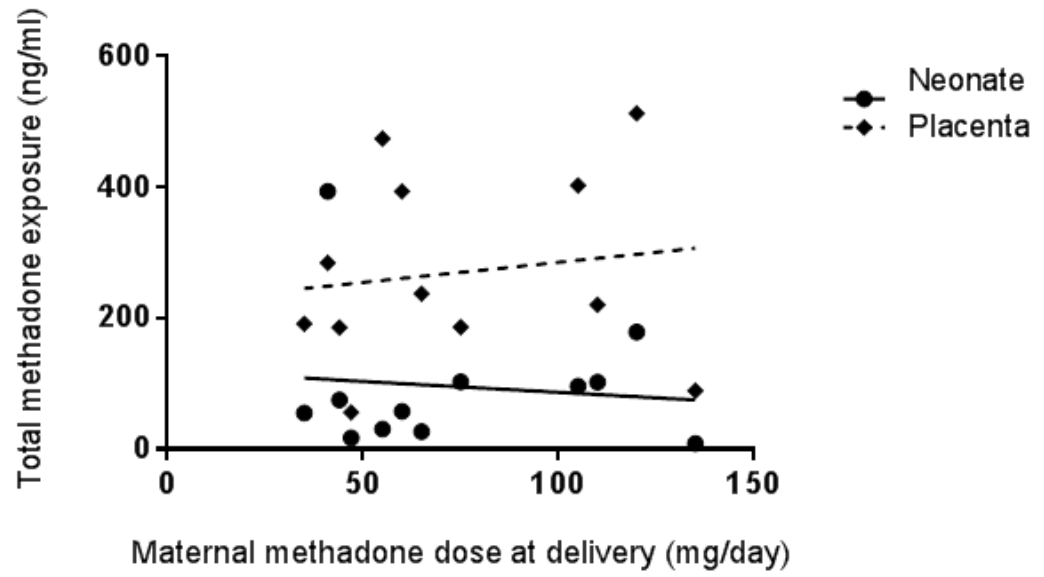


Figure 2.3. (S)-methadone metabolic ratio in the placenta and the neonate at 24 hours of life according to CYP2B6 genotype as measured by amount of metabolite divided by parent compound metabolite in ng per ml. Data is presented as mean \pm standard deviation.

S-EDDP/S-methadone
(ng/ml)

Placenta

Neonate

Table 2.5. Genotype prevalence and NAS severity markers where LOS is the median length of stay in hospital presented with the range. The need for treatment for NAS denotes the number of infants requiring morphine during their NAS management in hospital.

Gene	SNP:	Genotype:	N =	LOS (#days)	Need tx for NAS % (N)
		AA	14	3.5 (2-38)	36% (5)
	A785G (*4)	AG	2	2.5 (2-3)	50% (1)
		GG	2	29	100%
		CC	14	4 (2-29)	36% (5)
CYP2B6	C1459T (*5)	CT	2	2.5 (2-3)	0%
		TT	2	38	100% (2)
		GG	14	3 (2-38)	43% (5)
	G516T (*9)	GT	2	3 (2-4)	0%
		TT	2	29	100%
		AA	13	2 (2-29)	31% (4)
OPRM1	A118G	AG	5	8 (2-38)	60% (3)
		GG	0	NA	NA
		GG	8	3 (2-16)	38% (3)
COMT	val158met	GA	7	5 (2-38)	57% (4)
		AA	3	3 (2-4)	0 %

2.4.4 Discussion:

Methadone is a first line therapy for treating opioid dependency in pregnancy and throughout lactation. According to the American Academy of Pediatrics and the Centre for Substance Abuse Treatment methadone is considered safe and compatible during breastfeeding (10, 38). Controversial findings surrounding the association of maternal methadone dose at delivery and NAS severity (7, 22) was furthered in this cohort as maternal dose did not correlate with neonatal total methadone exposure, total methadone in the cord blood sample (placenta) or the need for pharmacologic management for NAS, indicating the potential importance of other clinical and genetic variables.

Prematurity has been associated with a reduced NAS severity due to an immature CNS, less accumulation of drug in fatty tissue and an overall decrease in length of exposure (7, 20). Further clinical risk factors for NAS potentially include gender, in which one study reported male babies with an greater need for pharmacologic intervention (21), maternal polysubstance use (20, 22), and duration of exposure. Prematurity was not assessed in this cohort, as only one neonate was born prior to 37 weeks. Table 2 revealed that low birth weight was associated with a reduced likelihood for requiring pharmacologic management which was unsupported by meta-analysis which revealed that smaller babies were more likely to be breastfed. This may have resulted from increased lactation encouragement from the clinical care team for infants of lower birth weight. In accordance with prior reports, breastfeeding was associated with less severe NAS in this cohort, as breastfed infants reported both a significantly shorter LOS and a decreased need for pharmacologic intervention (19, 55,

56). Previously reported infant serum methadone levels following exposure in breast milk are low (0.3-8.1ng/ml), thus breastfeeding should be encouraged for mothers maintained on methadone (without contraindications, such as HIV) (19, 57).

Predicting neonatal abstinence syndrome severity is a complex multifactorial problem and this small pilot study is the first of its kind to examine clinical and genetic risk factors and assess neonatal and placental methadone breakdown. This pilot study assessed the feasibility of prospectively collecting cord blood and newborn blood samples in mothers taking methadone during pregnancy. Cord blood samples were taken as a measure of placental exposure. Pilot studies are an essential component to the research process and provide information critical to the design of a larger scale study. Pilot study design strengths included a minimal amount of blood that was taken from the neonate. Sample collection was done in conjunction with other blood sampling, so that the baby will not experience any additional pain. In the future, it may be useful to collect additional blood samples at a later time point to assess the clearance of methadone as well as morphine metabolites in those infants requiring pharmacologic management. Limitations of this study included missing maternal genotypes and the lack of follow-up. Although initially planned in the study protocol, following several attempts all mothers were unable to be reached over the telephone. The authors recommend future studies seek consent to involve the mothers health care team (GP, paediatricians, social workers, public health nurses) that have more frequent contact with the patient to increase follow-up feasibility. Initiating pro-active follow-up techniques will increase compliance and data collection following discharge from hospital.

In conclusion this study demonstrated the feasibility of collecting cord blood and neonatal blood samples in babies exposed to methadone *in utero*. To the authors best knowledge this is the first report of the involvement of CYP2B6 polymorphisms in neonatal methadone metabolism and NAS severity. The strengths and limitations reported here should be used to design a future prospective cohort study to further investigate pharmacogenomic and clinical predictors of neonatal abstinence syndrome and enantiomeric methadone levels in the newborn.

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Chapter 3: Neonatal safety of codeine in breast milk

Part of this chapter has been published:

Kelly LE, Chaudhry SA, Rieder MJ, 't Jong G, Moretti ME, Lausman A, Ross C, Berger H, Carleton B, Hayden MR, Madadi P, Koren G. A clinical tool for reducing Central Nervous System depression among neonates exposed to codeine in breast milk. *PLoS-One* 2013; 8 (7): e70073

3.1 Codeine/morphine transfer into breast milk

For decades, codeine has been widely used in the management of post-partum pain. As previously discussed, studies have documented the transfer of both codeine and morphine into breast milk. The literature describes a neonate breastfed by a mother taking a low dose of morphine that showed serum morphine concentrations in the analgesic range of 4ng/ml but breast milk morphine levels varied from 10-100ng/ml (1). In a second neonate, a single 60mg codeine dose produced breast milk codeine concentrations ranging from 140-455 $\mu\text{g/L}$ during the first hour post administration (2). In the wild-type CYP2D6 metaboliser we would expect roughly 7% of a codeine dose to be converted to morphine. Following moderate codeine use (60mg, 4 times daily), analysis of milk samples from 11 healthy neonates revealed codeine breast milk concentrations between 33.8-314 $\mu\text{g/L}$ and morphine levels ranging from 1.9-20.5 $\mu\text{g/L}$ (3). The majority of these reports do not account for maternal genotype or for variability in the total length of time spent breastfeeding which may account for the high variability in both codeine and morphine breast milk levels.

3.2 Adverse neonatal effects following codeine exposure in breast milk

The first reports of adverse neonatal effects following maternal codeine use during lactation appeared in the literature in 1985 (4). In that report four neonates, whose mothers were taking 60mg of codeine every 4-6 hours, experienced apnea which resolved following the mothers' discontinuation of codeine (4). In 2006, a neonate succumbed to morphine poisoning following maternal use of codeine during lactation (5). Following an episiotomy

the mother of the healthy neonate was prescribed Tylenol #3 ® containing 30mg of codeine. Being prescribed a standard dose of two tablets twice a day; she subsequently reduced her intake to one tablet twice a day due to constipation and somnolence, which she continued for two weeks. A breast milk sample frozen on day 10 revealed morphine concentration of 87 ng/ml (5). On day 13 the neonate was found cyanotic and without vital signs (5). The mother's genotype revealed CYP2D6 UM status, resulting in an increased amount of morphine produced from a standard codeine dose. This fatality resulted in the FDA and Health Canada altering codeine labels to include a warning for nursing mothers (6).

In order to investigate further the correlation between maternal genotype and neonatal adverse events, a case-control study was initiated by the Motherisk Program at the Hospital for Sick Children (7). This retrospective study on 72 mother infant pairs identified neonatal CNS depression in 25% of babies and a significant correlation between adverse CNS symptoms in the mother and symptomatic infants (7). Mothers with the CYP2D6 UM genotype and the UGT2B7 *2/ *2 were at an increased risk for reporting adverse neonatal effects (7). In 2012, a report on 111 mother infant pairs by Sistonen et al., identified an 87% predictive value for codeine- related adverse neonatal effects, based on maternal dose, CYP2D6 and ABCB1 genotype (8). Also in 2012, a report by our group revealed a neonatal adverse event rate of 17% (35/210) following maternal codeine use (9). Limitations of these three retrospective studies include maternal self-report of neonatal effects and the potential for recall bias. However, we did use control groups of mother-child pairs exposed to only acetaminophen. As up to 50% of births are via caesarean section or episiotomy, and 80% of Canadian women initiate breastfeeding, the findings of these previous studies highlight the

importance of identifying strategies to potentially improve the safety of codeine use post-partum.

3.3 An intervention study for reducing Central Nervous System depression among neonates exposed to codeine in breast milk

3.3.1 Background and study rationale

Codeine-containing medications are commonly used for postoperative pain following caesarean section or episiotomy (10). Maternal breast milk is the ideal feeding method for newborns and is recommended by The American Academy of Pediatrics (11) and the World Health Organization (12). In Canada, up to 80% of new mothers initiate breastfeeding (13) and between 20-33% of all births are by caesarean section, rendering thousands of newborns exposed to codeine, and its active metabolites, morphine and morphine-6-glucuronide (M6G) through breast milk (14, 15).

Codeine is often considered a pro-drug, as the majority of its analgesic properties result from its biotransformation into morphine by the highly polymorphic cytochrome P450 enzyme 2D6 (*CYP2D6*). Morphine is further metabolized to the active morphine-6-glucuronide (M6G) and inactive morphine-3-glucuronide by UDP-glucuronosyltransferase (UGT) 1A1 and 2B7. The active metabolites of codeine, morphine and M6G, relieve pain via their action at the mu-opioid receptor. *CYP2D6* has over 80 variant alleles which differ in their contribution to enzyme activity (16). Individuals with a functional gene duplication,

resulting in the ultra-rapid metaboliser (UM) phenotype, have on average 50% higher plasma concentrations of morphine and M6G than the wild-type (17). The CYP2D6 UM phenotype in mothers has been associated with an increased risk for neonatal central nervous system (CNS) depression (7). Conversely, two alleles with no activity results in a poor metabolizer (PM) phenotype and these patients typically receive little or no therapeutic benefit from codeine as they form negligible amounts of the active metabolites (16, 17). Polymorphisms in drug transport by p-glycoprotein (*ABCB1*), the opioid receptor (*OPRM1*) and catechol-o-demethyltransferase are also thought to cause variability in response to codeine, morphine and their metabolites.

In 2006, our group reported a fatal case of CNS depression in a breastfed infant resulting from morphine overproduction in a CYP2D6 UM mother (5). Following the publication of this case, the United States Food and Drug Administration as well as Health Canada published public health advisories and label changes warning that codeine may not be safe during breastfeeding for infants whose mothers are CYP2D6 UMs due to an increase in morphine production (6, 18). Since then, a prospective study of women taking codeine postpartum revealed that 16.7% (35/210) of mothers reported CNS depression in their infants following postpartum use of codeine (9).

These risks of neonatal CNS depression led the Motherisk program to critically evaluate the available scientific evidence and propose guidelines for safe use of codeine during breast feeding (19). Further pharmacokinetic data confirmed that potentially toxic

morphine concentrations can be reached within 4 days in breastfed neonates after multiple doses of maternal codeine (20). The repeated observations of CNS depression after more than 4 day exposure to codeine through milk, the high concordance between maternal and neonatal CNS depression, and the typical adverse symptoms; including prolonged sleep, missing feeding, poor latch and poor weight gain were identified as clinical factors associated with infant CNS depression following codeine exposure (Figure 3.1)(19). The primary objective of the present study was to evaluate the effectiveness of these safety guidelines at improving neonatal safety.

3.3.2 Patients and methods:

This study was approved by the institutional Research Ethics Board at the University of Western Ontario and St. Michael's Hospital. Mothers taking codeine for pain following caesarean section at St. Michael's Hospital, Toronto, Canada were recruited between December 1, 2009 and November 30, 2011. A team member obtained written and informed consent from the expectant mother, as well as provided all study information and answering questions. At the time of their post-operative codeine prescription, women were provided with a copy of the codeine safety guidelines (Figure 3.1). At the time of recruitment, mothers were advised regarding the mode of action of codeine, effect of genetic makeup of mother on conversion of codeine to morphine. They were also informed about the possible adverse effects in mothers like sedation, dizziness, drowsiness, and constipation. Information was also given regarding the possible adverse effects on the breastfed neonates such as poor latching

onto the nipple or poor feeding, limpness, prolonged sleep or difficulty breathing. Mothers were given a 24 hour contact telephone number and were advised to bring their child to an emergency department if any of the above symptoms were noted. Due to the previously high reported rates of neonatal sedation 16% (35/210) it was deemed unethical to randomize a control arm without educational intervention (9).

Following delivery, provided it was unremarkable, the mother and infant normally remained in hospital for several nights before being discharged home. In the 16-hour period after delivery the mother was not typically given any analgesic medication except for epidural analgesia. After 16 hours most mothers began taking Tylenol #3 (500 mg acetaminophen and 30 mg codeine) for their pain every 4-6 hours as needed and were sent home with a prescription for this medication or other non-opioid analgesic(s). All mothers were given a "Patient Medication and Breastfeeding Tracking Sheet" to track breastfeeding progression, medication use and infant health. One week after discharge, a follow-up telephone interview was conducted to assess maternal and infant health with a specific focus on neonatal CNS depression. Follow-up was conducted using a standardized questionnaire to ensure all participants provided the same quantity and quality of follow-up data. In order to identify neonatal sedation data was collected on the several parameters including the number of times an infant fed per night, whether or not they woke up for feeds and the length of time per feed. At the time of recruitment mothers were advised to look for any latching problems, breathing difficulties, or any fluctuations in skin colour which was reported at the time of follow-up. Data was also collected on the number of bowel movements per 24 hours, the length of sleep at one time and the total amount of sleep per night. The interview also included maternal

satisfaction with pain control, self-reported analgesic dose, and any maternal adverse drug reactions.

Mothers had the option of providing a saliva sample for genetic screening. The saliva sample was labelled with a unique barcode and couriered to the Canadian Pharmacogenomic Network for Drug Safety (CPNDS) Core Laboratory in Vancouver, British Columbia. In order to determine the role of genetic variability on the codeine and morphine pathways, polymorphisms in several key genes (ABCB1, COMT, UGT 2B7, OPRM1) were analysed. Maternal DNA was purified from saliva samples using the QIAmp DNA purification system (Qiagen, Toronto, ON, Canada) according to the manufacturer's protocol. DNA samples were genotyped for variants in *CYP2D6* using the AutoGenomics INFINITI® Analyzer and the *CYP450 2D6I* Assay (AutoGenomics Inc., Vista, CA, USA), as well as SNaPshot, and TaqMan copy number assays as previously described (8, 21). A genotype activity score was calculated based on the scores of the individual alleles and patients were classified into four *CYP2D6* phenotype classes: poor metabolizers (PM: activity score of 0); intermediate metabolizers (IM: activity score 0.5-1); extensive metabolizers (EM: activity score 1.5-2; and ultra-rapid metabolizers (UM: activity score >2 due to a functional gene duplication). *CYP2D6**1 or *2 alleles were assigned an activity score of 1, the partially functional *9, *10, *17, *29, or *41, alleles were assigned an activity score of 0.5, and the non-functional *3, *4, *6, *7, *8, *12, or *14 alleles were assigned an activity score of 0. Whole gene deletions (*5) were assigned a score of 0. Activity scores in the case of whole gene duplications were assigned according to the number of functional *CYP2D6* copies. If none of the

aforementioned alleles were detected, the individual was assigned the default wild-type allele of *1. Gaedigk and colleagues describe in more detail the *CYP2D6* alleles (22).

DNA samples were also genotyped for genetic variations in the *ABCB1* gene: 61 A>G (rs9282564), 1236C>T (rs1128503), 2677G>T/A (rs2032582), and 3435C>T (rs1045642), *COMT* (rs165815, rs4633, rs4818, rs740602) and *OPRM1* gene 118 A>G (rs1799971) using TaqMan® genotyping assays (Applied Biosystems, Foster City, CA, USA), as previously described (8).

Continuous parametric data were compared using an unpaired Students t test and are reported as mean values with the corresponding standard deviation. Nonparametric continuous data are reported as median (range) where appropriate and were analysed using the Mann Whitney U test. Normally distributed data were compared by Student's t test for unpaired results. All discrete and binary data is conveyed in percentages and significance was tested using a Fisher Exact test.

Figure 3.1. Safety guidelines for codeine use during breastfeeding. Motherisk safety guidelines for codeine use in breastfeeding pamphlet given to all expectant mothers with planned caesarean sections at St. Michaels Hospital in Toronto, Ontario, Canada (19). Women were advised to take codeine for as short a period as possible (3-4days postpartum) and were advised to seek the care of a physician if they required pain medication beyond this point. Mothers were also advised to breastfeed before taking codeine to maximize the time to eliminate codeine in between feeds. ©Motherisk Program and The Hospital for Sick Children. Reprinted with permission from the Canadian Family Physician

Motherisk guidelines for safe use of medications that contain codeine during breastfeeding

- In most cases, the occurrence of CNS depression is consistent between the mother and the baby. If the mother suffers from symptoms of CNS depression (eg, somnolence, grogginess), a physician should examine the baby for signs of CNS depression as well.
- If the baby is not fed well, does not gain wt, does not wake up to be fed, or shows limpness he or she should be examined by a physician.
- CNS depression in the baby appears to worsen after 4 days probably owing to the accumulation of morphine with more breastfeeding. If possible codeine should not be used for longer than 4 days. If pain still necessitates codeine an attempt should be made to decrease the dose or to switch to non-codeine painkillers (NSAIDs).
- Women who convert more codeine to morphine have a duplication of the gene encoding for cytochrome P450 2D6. This genetic predisposition can be detected by genetic test. This test although not available in most hospitals is available on the market.

3.3.3 Results

A total of 255 women consented to participate in this study, however 15 were lost to follow-up and one woman withdrew for personal reasons. There were 268 women approached to participate in the study. We included 238 women giving birth to 239 healthy babies (129 males, 108 females and one set of male/female twins). Ethnicity was self-described, and the mother's grandparent's country of origin were documented. There were 87 women that identified as Caucasian (37%), 42 as South or Central American (18%), 36 as Asian (15%), 34 as African American (14%), 24 identified as Indian (10%) and the remaining 15 originated from other countries. Saliva samples were collected from 192 participants.

Expectant mothers' mean weight was 74.86 +/- 12.10 kg prior to delivery with a mean age of 32.82 +/- 5.58 years. Following delivery mothers remained in hospital for a mean of 1.91 +/- 0.75 days. The mean dose of codeine taken by the participants was 1.18 +/- 0.54 mg/kg/day for 2.56 +/- 1.51 days. When asked why they stopped taking codeine, 86% (204/237) reported that they had no more pain, 7% of mothers that stopped taking codeine due to adverse reactions in themselves (10/237 for excessive sedation, 6/237 for constipation,). Six mothers, (2.5%) discontinued codeine use over concerns for their infants and 4.6% stopped taking codeine because they found it ineffective in managing their pain. Patient's genotype did not correlate with the reasons for codeine discontinuation. There was only one mother who contacted the 24 hour hotline.

Five women reported sedation in their child following exposure to codeine in breast milk (2.1%, 5/238). None of these five mothers reported adverse reactions to codeine in themselves during use of the drug. There were no significant differences identified in the length of time spent breastfeeding, or the total amount of codeine used (Table 3.1). Women reporting sedated infants were taking codeine for a significantly longer period of time, and on average (4.80 days) in excess of the 4 days recommended by the guidelines (4.80 ± 2.59 days vs. 2.52 ± 1.58 days, $p = 0.0018$ by Student's t test). There were no sedated infants or maternal adverse drug reactions reported in the CYP2D6 ultra-rapid metabolizer group. Mothers with CYP2D6 UM status breastfed for a similar length of time and took similar amounts of codeine as all remaining phenotypes (Table 3.2). A total of 9% (19/238) of mothers reported adverse reactions following codeine use postpartum. These complaints included sedation, dizziness, constipation and nausea. Maternal adverse response did not correlate with neonatal sedation (0/19 vs. 5/219). All mothers with symptomatic neonates were non-Caucasian in origin.

Maternal DNA was analyzed for polymorphisms in ABCB1, COMT, UGT 2B7, OPRM1. Maternal adverse events did not correlate with any of the genes analysed in this study (Table 3.3). Furthermore there were no associations of infant sedation with any of the genes evaluated. The minor allele frequency (MAF) for all polymorphisms was not significantly different from the global reported MAF as taken from the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

Table 3.1. The influence of clinical factors on neonatal sedation (Infant ADR). Demographic characteristics of Mothers of sedated infants (Infant ADR) and those whose infants were did not report any changes in health status (asymptomatic). Statistical significance was set at $P < 0.05$. Parametric values are presented as mean \pm standard deviation and nonparametric values are presented as median (range).

	(N = 5)	(N = 233)	
	Infant ADR	Asymptomatic	P Value
No. days taking codeine	4.8 ± 2.6	2.5 ± 1.6	0.01
Total amount of codeine used (mg/kg)	5.1 ± 2.4	3.2 ± 2.5	0.08
No. times feeding per day	8.5 (7.0-9.0)	9.0 (3.0-13.0)	0.27
Baby GA	38.6 ± 1.1	39.1 ± 1.4	0.44
Baby Birth Weight (g)	3392.4 ± 584.4	3403.1 ± 510.12	0.96
No. consecutive hours slept	3.5 (2.5-4.0)	2.5 (1.5-4.5)	0.19
Maternal ADR	0	8 % (19/242)	0.67
Formula supplementation	20 % (1/5)	17 % (39/229)	0.41
Non-Caucasian	100 % (5/5)	62 % (143/230)	0.10

Table 3.2. The influence of CYP2D6 genetic factors and breakdown of demographics. Patient characteristics and reported outcomes broken down by Maternal CYP2D6 phenotype, where a poor metabolizer has a CYP2D6 activity score of 0, intermediate a score between 0.5-1.0, extensive a score between 1.5-2.0 and ultra-rapid a score of 2.5 or higher. Data are presented as mean \pm standard deviation, and median (range) for nonparametric data.

	Poor Metabolizer (N = 11)	Intermediate Metabolizer (N = 70)	Extensive Metabolizer (N = 105)	Ultra-rapid Metabolizer (N = 6)
Total Codeine Dose (mg/kg/ day)	1.4 ± 0.6	1.1 ± 0.4	1.2 ± 0.5	1.2 ± 0.3
Total No. days on codeine	2 (1-3)	2 (1-9)	2 (1-9)	2 (1-3)
Maternal Age (years)	34.5 ± 5.5	32.9 ± 5.7	32.6 ± 5.51	32.0 ± 6.8
No. times breastfed in 24hours	9 (7-10)	9 (6-13)	8.75 (3-11.5)	9 (7-13)
Baby GA	39.3 ± 1.4	39.0 ± 1.2	38.5 ± 1.3	38.8 ± 0.4
Baby weight (grams)	3517.5 ± 513.4	3379.4 ± 510.8	3421.3 ± 519.5	3199 ± 321.8
No. reported infant ADRs (%)	0	3 (4 %)	2 (2 %)	0
No. reported maternal ADRs (%)	1 (9 %)	7 (10 %)	7 (6 %)	0

Table 3.3. The influence of genetic factors on reported maternal adverse effects. Minor allele frequency (MAF) for polymorphisms in the p-glycoprotein transporter (ABCB1), catechol-o-methyltransferase (23), mu-opioid receptor (OPRM1) and UDP glucuronosyltransferase (UGT) 2B7 in mothers reporting adverse drug reactions (ADR) in themselves compared to those that were asymptomatic (healthy). Significance value was set at $P < 0.05$.

Gene	SNP	Global MAF:	Observed MAF (N):	Allele	Percent of mothers reporting ADRS (N = 19)	Percent of healthy mothers (N = 218)	P Value
ABCB1	rs1128503	0.42	T = 0.445 (212)	TT	21	25	0.21
				TC	37	44	0.16
				CC	42	31	0.12
	rs2032582	0.34	A = 0.21 (100) T = 0.21 (98)	AA	21	16	0.19
				AT	5	6	0.38
				GG	32	39	0.16
				GT	37	35	0.19
				GA	5	3	0.38
				TT	26	21	0.19
	rs1045642	0.40	T = 0.44 (207)	TC	32	45	0.10
CC				42	34	0.15	
TT				26	21	0.19	
COMT	rs4680	0.39	A = 0.43 (204)	AA	21	21	0.23
				AG	32	46	0.10
				GG	47	33	0.10
	rs4633	0.39	T = 0.42 (200)	CC	47	34	0.10
				CT	37	45	0.15
				TT	16	21	0.27
	rs4818	0.32	C = 0.65 (306)	CC	37	41	0.18
				CG	42	48	0.17
				GG	21	11	0.12
OPRM1	rs1799971	0.19	G = 0.22 (105)	AA	68	62	0.18
				AG	32	30	0.20
				GG	0	8	0.23
				CC	37	36	0.20
UGT 2B7	rs7439366	0.47	T = 0.44 (206)	CT	37	40	0.19
				TT	36	24	0.21
				CC	37	36	0.20

3.3.4 Discussion

Given the considerable genetic variability in codeine response, its frequent use postpartum, and the previously reported high rates of neonatal sedation, it was imperative to evaluate the effectiveness of a clinical tool to improve infants' safety. We have developed safety guidelines that combined clinical data from two case control studies with previously identified genetic markers of toxicity (6) (7) . The present study is the first prospective study to evaluate the effectiveness of codeine guidelines for breastfeeding mother/infant pairs. The safety guidelines developed recommend use of codeine for no longer than four days postpartum, as generally milk production is lower in these first few days of life, and thus neonatal morphine accumulation is minimized (19, 20). The only significant difference seen between symptomatic and nonsymptomatic infants in the present study was the duration of codeine use, where neonatal CNS depression occurred among infants exposed to codeine for more than 4 days. These data suggest that minimizing codeine use to the first four days can significantly reduce neonatal exposure and reduce the incidence of reported neonatal CNS depression.

Based on current codeine warnings, ethical considerations precluded the randomization of a group of mother-child pairs to not receive counselling on the potential neonatal risks of codeine use thus preventing a true control group. Using the Motherisk Codeine Safety Guidelines (19), the present cohort reported considerably lower rates of neonatal sedation [2% (5/238)] than a previously reported rate in a prospective study [16%

(35/210)] (9). The lack of formal randomized control arm in this trial does introduce some limitation in data interpretation as selection criteria and the time of follow-up is variable between studies. When following the guidelines mothers were less likely to supplement infant feeding with formula (16.74%) which was reported at 57.06% in a previously prospective cohort of women breastfeeding while taking codeine post-caesarean section (9). Providing mothers with the education tools to make informed decisions regarding their choice to breastfeed is important as maternal breast milk provides optimal nutrition for neonates.

This study further supports educating mothers on the symptoms of codeine toxicity in their infants and the importance of a prompt response if these symptoms appear. The guidelines also recommend that if a woman is still in pain after 4 days of codeine use, she should attempt a non-opioid analgesic such as ibuprofen (19). In this study 86% of women reported that they discontinued codeine because their pain was well managed before 4 days. This suggests that codeine is effective and safe in treating maternal pain following caesarean section when used according to the Motherisk guidelines for no more than four days.

There are several genetic polymorphisms important in determining codeine and morphine response. Previous work has identified maternal genetic factors that increase the risk for sedation in neonates exposed to codeine through breast milk including CYP2D6 UM phenotype, *UGT2B7**2/*2 and *ABCB1* 2677TT (7, 8). The *UGT2B7* C802T variant which gives rise to a *UGT2B7* *2 phenotype has shown to increase production of M6G, which is several fold more potent than morphine (24). A large portion of the original codeine dose is

also glucuronidated by *UGT2B7* into codeine-6-glucuronide. This polymorphism was associated with an increased risk for neonatal sedation when found in combination with the CYP2D6 UM phenotype; however this finding has not been repeated (7). The increased morphine production seen with the CYP2D6 UM phenotype has been associated with an increased risk for codeine related adverse events in both mother and infant (7). Of importance, while following the safety guidelines, there were no maternal or neonatal adverse events associated with the CYP2D6 or UGT 2B7 genotype (Table 3.2). There is ethnically determined variability in polymorphism frequency that should be evaluated in future studies. For example, the frequency of the CYP2D6 UM phenotype has been reported to be as high as 40% in women of North African descent, which is only described in approximately 2% of Caucasians (25). In this cohort none of the mothers of symptomatic infants conveyed the CYP2D6 UM phenotype, however, all five women identified themselves as non-Caucasian. While it is important to independently study the effects of ethnicity on codeine response, the present cohort represents the diverse patient populations typically seen in many North American Institutions.

The genetic polymorphisms responsible for the production of morphine are not the only sources responsible for the variability seen in codeine response. Single nucleotide polymorphisms (SNP) in P-glycoprotein (*ABCB1*) have been associated with increased central adverse effects in patients taking morphine (26). This efflux transporter protein plays an important role in transporting codeine and morphine out of the brain. The maternal *ABCB1* 2677TT polymorphism is significantly associated with neonatal CNS depression following breastfeeding resulting from a decrease in efflux out of the blood brain barrier (8).

Conversely, a seemingly protective SNP in the mu-opioid receptor (OPRM1) A118G decreases receptor expression in a lower incidence of opioid toxicity (27). Catechol-o-methyltransferase (23) is responsible for the breakdown of neurotransmitters and interacts with the opioid receptor. Increased morphine sensitivity has been associated with several COMT SNPs (28). Polymorphisms in maternal OPRM1 and COMT have not been associated with an increased risk for neonatal sedation following breastfeeding but their importance for morphine effectiveness warrants further investigation. Currently these polymorphisms are not routinely screened for before a patient is given codeine for pain.

Our study shows that post-partum safety guidelines may improve the safety of codeine exposure in virtually all breastfed infants regardless of their genetic profile. Alternatively, post-partum maternal analgesia with Nonsteroidal anti-inflammatory agents (NSAIDs) should also be considered, as a recent systematic review of all randomized trials suggests that codeine is not superior to NSAIDs for analgesia after laparotomy (29). In conclusion, in our study mothers following the safety guidelines reported low levels of neonatal sedation (2%). Maternal adverse event rates were low, even in those genetically determined to be of high risk while maternal pain was effectively managed. These guidelines present a simple and effective approach to improving the neonatal safety while maintaining effectiveness of postpartum codeine use.

3.4 References

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Chapter 4: Post-operative opioid use in children with obstructive sleep apnea

Part of this chapter has been adapted from published work:

Kelly LE, Rieder M, van den Anker J, Malkin B, Ross C, Neely M, Carleton B, Hayden M, Madadi P, Koren G. More codeine fatalities after tonsillectomy in North America. *Pediatrics* 2012; 129 (5) e1343-1347

Part of this chapter has been submitted for publication:

Kelly LE, Sommer DD, Ramakrishna J, Hoffbauer S, Arbabtafti S, Maclean J, Reid D, Koren G. A randomized trial comparing morphine and ibuprofen for post-tonsillectomy pain management in children with sleep disordered breathing. Submitted to NEJM February 19, 2014

4.1 Sleep apnea definition, prevalence and treatment in children

Pediatric obstructive sleep apnea syndrome (OSAS) results from an increase in upper airway resistance or prolonged airway obstruction that disrupts ventilation and alters breathing patterns during sleep (1). OSAS is the most severe form of sleep disordered breathing in childhood. OSAS affecting 2-3% of all children from birth to adolescence, induces hypoxic state which can alter gas exchange and cellular respiration (2). According to the most recent census data approximately 600,000 - 1,800,000 North American children under the age of 15 are affected by OSAS (3, 4). The most common cause of pediatric OSAS is hypertrophy of the adenoids and/or tonsils. Untreated OSAS can cause several long-term sequelae including increased aggression, depressed mood, nocturnal enuresis, systemic hypertension and delayed physical growth (2).

During sleep, children with OSAS have recurrent episodes of full or partial airway obstruction resulting in hypoxemia, hypercapnia, and sleep disruption (5). Diagnostic criteria for pediatric OSAS include an apnea-hypopnea index (AHI) greater than one event per hour and nadir oxygen saturation below 92% (6). Upon diagnosis by polysomnography, the primary treatment for pediatric OSAS is adenotonsillectomy (2). While the many children improve their sleep apnea after surgery, up to 33% may not be cured (7-9). Moreover, after surgery, improvement may not occur for days to weeks, and many patients experience respiratory events in the peri- and post-operative period (10). The post-surgical pain associated with this procedure is moderate to severe and has been commonly managed in North America by opiates, mostly codeine (11, 12).

4.2 Pain management following surgery

The pain associated with AT is classified as moderate to severe and due to a fear of increased tonsillar bleeding many physicians are hesitant to prescribe non-steroidal anti-inflammatory medications (NSAIDs) (13). Commonly, the opioid codeine was given to young children post-AT (13). Codeine is a prodrug, whose analgesic properties are dependent on its conversion to morphine. The metabolism of codeine to active morphine depends on the highly polymorphic *CYP2D6* pathway. Identified polymorphisms in this gene have given rise to the definitions of poor (PM), extensive (EM) and ultra-rapid (UM) metaboliser phenotypes resulting in varied amounts of morphine produced from a standard codeine dose. In the general population approximately 10% of codeine is bioactivated to morphine, however when administered to a poor metaboliser, almost no morphine is produced (14, 15). A functional gene duplication resulting in a *CYP2D6* UM shows a gene-dose effect; as the number of *CYP2D6* gene copies increases, so does the amount of codeine converted to morphine. A patient with the *CYP2D6* UM can produce 50% up to 75% more morphine than a *CYP2D6* EM (15).

The use of NSAIDs medications in children post-tonsillectomy is controversial due to their potential to adversely affect platelet function, resulting in prolonged bleeding (13). Hence, these medications are not used by many surgeons in North America. In a randomized double-blind pilot study, children receiving ibuprofen required medication for a longer period of time to treat pain than those who had received acetaminophen with codeine (13). In addition, ibuprofen was associated with a 12.5% postoperative hemorrhage rate, as compared

to 0% in the codeine group. These authors concluded that acetaminophen with codeine is safer and more efficacious than ibuprofen in the management of post-tonsillectomy/adenotonsillectomy pain in children (13). In contrast, a Cochrane review of over 1000 children found that NSAIDs (ketorolac excluded) did not significantly increase post-tonsillectomy bleeding when compared to placebo or other analgesics (odds ratio = 1.46, 95% confidence interval = 0.49-4.40) (16). A recent systematic review and meta-analysis by our group comparing bleeding rates following post-tonsillectomy use of NSAIDs vs placebo or opioids included 1747 children and 1446 adults (17). In the large population of children studied there was no evidence for an increase tonsillar bleeding risk following the use of NSAIDs (odds ratio = 1.06, 95% confidence interval = 0.65-1.74) (17).

4.3 Case report: More codeine fatalities following tonsillectomy in North American children

The purpose of this report is to discuss three previously unreported severe cases of opioid induced toxicity in children with OSAS post-AT. Consent was received from the Coroner's office in Cases 1 & 3 and from the parents in Case 2.

Case 1: At a regional hospital in Northern Ontario, Canada, a 4 year old (27.6 kg) First Nations' boy underwent AT for OSAS and recurrent tonsillitis. He was discharged home after an uneventful overnight stay on liquid codeine at an age appropriate dose (8 mg per dose, up to 5 doses a day PRN) (18). His parents reported him to be sedated and lethargic the day after hospital discharge. The next afternoon, following a total of 4 codeine doses he was brought to

hospital without vital signs. His post-mortem morphine serum concentration was 17.6 ng/mL (therapeutic morphine range 4.5 ± 2.1 ng/mL) (11). His toxicology screen revealed a blood codeine level in the expected range following therapeutic use and there were no other medications detected. Genotyping revealed a gene duplication and a *CYP2D6* UM phenotype (*CYP2D6* *1/*2A_xN). His UM *CYP2D6* status resulted in an increased morphine leading to respiratory arrest. Post-mortem analysis revealed the cause of death to be bilateral acute bronchopneumonia, and morphine toxicity following adenotonsillectomy.

Case 2: A 3 year old girl (14.4 kg) of Middle Eastern descent underwent tonsillectomy for OSAS and was discharged after a 24-hour hospital stay at a Canadian Children`s Hospital. In hospital she received two doses of codeine syrup (15mg each). Upon discharge she was given a combination of codeine and acetaminophen (15 mg codeine/150 mg acetaminophen) every 4-6 hours PRN. More than 6 hours after her final codeine dose (total 60 mg codeine) she was found unresponsive with a fever of 100° as measured at home. Upon admission to hospital she presented with minimal respirations and an oxygen saturation of 65%. She experienced one bout of vomiting with mild-dark blood observed. Her blood morphine concentration measured 17 ng/mL. Following successful resuscitation, mechanical ventilation and naloxone dosing (1.5mg) she showed a prompt improvement in her symptoms. The next day she was extubated and recovered fully. Her genotype was determined to be an extensive metaboliser (EM) (*CYP2D6* *1/*1). In this case, her morphine levels suggested ultra-rapid metabolism, which was not consistent with her genotype. However, the EM genotype often overlaps with the UM phenotype (14, 19).

Case 3: A 5 year old boy (29 kg) underwent bilateral myringotomy tube placement, and AT for recurrent tonsillitis and snoring in the Southern United States. Following surgery he was prescribed acetaminophen and codeine (12 mg codeine) every 4 hours. This total 72 mg/day is within the recommended range of 6 mg/kg per day (20). The child was released home, but was found without vital signs by his mother 24 hrs after his surgery. The autopsy did not reveal a cause of death. This child's post-mortem codeine concentration was 79 ng/mL, and morphine concentration was 30 ng/mL. A pharmacokinetic model using Pmetrics software (Los Angeles, United States) was constructed based on published pediatric pharmacokinetic characteristics to simulate expected time concentration profiles for codeine and morphine based on his age, weight, and dosing schedule (Appendix 1) (21, 22). Finally, we compared his measured codeine and morphine concentrations to the expected ranges.

The measured codeine concentration of 79 ng/mL approximately 8 hours after his last dose is at the 56th percentile of predicted pediatric codeine concentrations. In contrast, the measured morphine concentration of 30 ng/mL is at the 99th percentile of predicted concentrations at the normal pediatric rate of conversion. The codeine levels are consistent with his prescription of 0.41 mg/kg every 4 hours PRN, which is within the recommended dose according to the published prescribing recommendations (20). It is highly likely that the child was a *CYP2D6* UM based on his exceedingly high morphine concentration relative to codeine.

Discussion:

In 2009 our group first reported fatal codeine toxicity in toddler with the *CYP2D6* UM phenotype following AT (23). We now describe here two deaths and one severe case of apnea

among young children administered standard doses of codeine. These children had morphine levels in the range associated with CNS depression, apnea and death. While 70-80% of children undergoing AT for OSAS improve their apnea long-term, many children's respiratory condition worsens immediately after surgery (24). It is conceivable that among children in whom the apnea was not resolved after surgery, morphine, as a powerful CNS depressant, may further worsen the respiratory condition. Children with a *CYP2D6* UM phenotype, have increased risk of serious CNS depression and apnea (23). The *CYP2D6* UM status occurs in roughly 1-10% in individuals of European descent, but in up to 30% of North African descendants (11). Genetic testing revealed UM status in Case 1 and the use of metabolic ratios suggests that Case 3 followed the same genetic pattern. Furthermore between 6-10% of the Caucasian population are poor *CYP2D6* metabolizers (2). In these individuals the ability to convert codeine to the active morphine is reduced, resulting in therapeutic failure and poor pain relief. The elevated morphine levels seen in the *CYP2D6* EM genotype, as was detected in Case two, often overlap with the UM phenotype (14). The assay used in these case reports has several complexities described by Madadi et al. (25). While the Caucasian *CYP2D6* alleles have been well studied, single nucleotide polymorphisms in the First Nations population are not well characterized. It is therefore possible that the child in Case 1 possesses a rare non-functional allele undetected by the assay.

In two of the cases described here, despite overnight care in hospital, the child's sleep apnea critically worsened at home. These children were prescribed age-appropriate codeine doses and were taking their medications in accordance with the published dosing guidelines (18). This suggests that a one night follow-up in hospital may not be able to effectively detect all children at increased risk of severe respiratory complications. Since *CYP2D6*

polymorphisms are not routinely screened for prior to prescribing codeine, sending these children home without conclusive observations can mean that a high-risk patient may go unnoticed. The children presented in these cases were receiving doses within the recommended weight-adjusted dose of 0.5-1-mg/kg q4-6 hours (maximum 6mg/kg per day) (8). Of potential importance, the children in Cases 1 & 3 were significantly overweight (97th percentile) (26). As morphine sparsely distributes to fatty tissue, dosing based on total body weight instead of lean mass could have partially contributed to morphine accumulation. Post-mortem blood samples in Case 1 & 3 were obtained approximately 14hrs and 8hrs after the last codeine dose. It is possible that some post-mortem morphine redistribution out of organ and muscle compartments into the blood occurred, however in such a short time this is not thought to have been a major contributor. In Case 2, serum samples were taken immediately upon her presentation to the emergency department, 6 hours after her last dose. The active morphine-6-glucuronide metabolite is eliminated renally (27). However, it is unlikely that the glomerular filtration rate was impaired in these children who were all healthy prior to surgery.

Pediatric OSAS is a common condition which presents high rates of analgesic complications for post-operative pain management. These three cases strongly suggest that codeine, and potentially other opioids metabolized by the *CYP2D6* pathway, cannot be considered safe analgesics for young children after AT for OSAS.

4.4 FDA Investigation and black box warning

On August 15, 2012, following publication of the previously described case reports, the Food and Drug Administration (28) issued a Drug Safety Communication: Codeine use in certain children after tonsillectomy and/or adenoidectomy may lead to rare, but life-threatening adverse events or death (29). This announcement summarized the case reports, provided details surrounding the increased metabolism of codeine to morphine with the ultra-rapid metabolizer phenotype, as well as provided the prevalence in different ethnicities (29). Information was provided to parents and care givers regarding signs and symptoms of morphine toxicity (sleepiness, confusion, difficult or noisy breathing) and to stop giving codeine and seek emergency medical attention. Health care professionals were advised to counsel parents on the signs of morphine toxicity and to consider prescribing alternative analgesics post-tonsillectomy and to report and adverse events involving codeine to the FDA MedWatch program (29). The FDA also announced a further review to identify additional cases of overdose or death following codeine use in children and if these events occurred during post-operative pain management.

Following a six month investigation the FDA released a “Safety review update of codeine use in children; new *Boxed Warning* and *Contraindication* on use after tonsillectomy and/or adenoidectomy” on February 20, 2013 (30). This update issued the FDA’s strongest warning, adding a Boxed Warning to codeine-containing products about the risks of codeine use in children post-adenoid/tonsillectomy. Following the August Safety Communication further investigation revealed 13 cases of death or life-threatening overdose in children

receiving standard, appropriate codeine doses (30). These cases included children from 21 months – 9 years of age and the majority (8/13) were post-adenotonsillectomy. Furthermore three cases involved codeine use following respiratory tract infections (30). A contraindication issued by the FDA is a formal recommendation to restrict codeine use in this population.

While the Boxed Warning suggests avoiding codeine for post-adenotonsillectomy pain management in children they do not make recommendations as to safe and effective alternate analgesic in pediatric patients. The analgesic options in children with obstructive sleep apnea are similar to other surgical patients, there is however a smaller margin for error due to the higher potential for respiratory complications following surgery (31). Further complicating the physician's analgesic choice is that the removal of adenoids and tonsils is only curative in approximately 65% of children with obstructive sleep apnea and the addition of a CNS depressant may further worsen the child's respiratory condition (7, 8).

4.5 Investigating the safety and effectiveness of morphine and ibuprofen in children post-adenotonsillectomy for obstructive sleep apnea

4.5.1 Background and rationale:

Sleep disordered breathing (SDB) is characterized by a disruption in ventilation and breathing patterns during sleep. SDB ranges in severity from snoring to obstructive sleep apnea (32). During sleep, children with SDB have recurrent episodes of full or partial airway obstruction resulting in hypoxemia, hypercarbia and sleep disruption (5). In children, SDB is often caused by hypertrophy of the tonsils and/or adenoids and is commonly managed by tonsillectomy with or without adenoidectomy (2). The pain associated with this procedure is moderate to severe and over 500,000 tonsillectomies are performed on children in the US every year (32, 33). Clinical practice guidelines for post-tonsillectomy pain management recommend educating parents on assessing pain in their children, maintaining proper hydration, and maintaining adequate analgesia, especially in the first few post-operative days (32).

Until recently, codeine was considered a first line analgesic for post-operative treatment. However, on August 15, 2012, following the publication of three codeine related fatalities post-tonsillectomy, the Food and Drug Administration (28) issued a Drug Safety communication advising practitioners that codeine use in certain children after tonsillectomy and/or adenoidectomy may lead to rare, but life-threatening respiratory failure and death.

These children often have an ultra-rapid CYP 2D6 genotype, leading to excessive production of morphine from codeine. Further investigation revealed 13 cases of death or life-threatening overdose in children receiving standard, appropriate codeine doses and the majority of these cases (8/13) were related to tonsillectomy patients (30).

While the Boxed Warning suggests avoiding codeine for post-tonsillectomy pain management, no recommendations have been made as to safe and effective alternate analgesic in pediatric patients. Due to a fear of increased bleeding, many surgeons have been hesitant to prescribe non-steroidal anti-inflammatory drugs (NSAID). Our recent meta-analysis', including over 1700 children, did not detect an increased risk of bleeding following post-tonsillectomy NSAID use (17). The contraindication of codeine for post-tonsillectomy analgesia has resulted in a shift to oral morphine, as unlike codeine, the metabolism of morphine is not associated with large variability in toxic risk. Presently, the safety and effectiveness of both ibuprofen and morphine in this population is unclear. The objective of this randomized clinical trial is to assess the safety and effectiveness of post-tonsillectomy analgesia with morphine and ibuprofen in children with SDB.

4.5.2 Patients and Methods:

After approval by McMaster University, The Hospital for Sick Children and The University of Western Ontario Research Ethics Boards, families were invited to participate in a randomized-controlled trial, where their children would receive oral acetaminophen and

either morphine or ibuprofen for pain management, following tonsillectomy +/- adenoid removal. These two groups were run in parallel with recruitment at the McMaster University Medical Centre in Hamilton, Ontario with an allocation ratio of 1:1. This trial was registered with ClinicalTrials.gov (NCT01680939) and there were no changes made to the methodology following the trial commencement. The inclusion criteria consisted of children with sleep disordered breathing aged 1-10 years, scheduled for tonsillectomy, with or without adenoidectomy. In addition to parental consent, children above the age of 7 years were asked to complete an assent form, acknowledging their willingness to participate. Children were excluded if they had previously undergone (adeno)tonsillectomy, had asthma, obesity (BMI >30), craniofacial/neuromuscular/haematological/cardiac abnormalities or contraindications to general anesthesia.

Sleep disordered breathing encompasses a variety of abnormal breathing patterns with OSA falling toward the more severe end of this spectrum. Although polysomnography (PSG) is the gold standard in diagnosis of pediatric OSA, fewer than 10% of pediatric tonsillectomy patients undergo the study (34). Consensus regarding the diagnostic criteria for pediatric OSA remains elusive (35). Guidelines developed by the American Academy of Otolaryngology recommend routine PSG only for patients with complex medical conditions including obesity, Trisomy 21, craniofacial abnormalities, and neuromuscular/metabolic disorders. As well, polysomnography prior to tonsillectomy should be obtained if the need for surgery is uncertain or when there is discordance between tonsillar size and suspicion of sleep disordered breathing on history. In the current study, patients with sleep disordered breathing and/or apneic episodes on history were evaluated preoperatively with home overnight oximetry (36).

At the pre-operative appointment, parents were provided with a pulse oximeter (Nellcor-Boulder N-600) including Oximax Max-p sensors to take home. The research team instructed parents how to properly apply the oximeter to the child the night before surgery. Parents were also instructed to repeat overnight oximetry measurements the night following surgery. The oximeter was used to monitor respiratory parameters during sleep. The primary outcome variable was changes in respiratory parameters (O_2 saturation and number of apnea events per hour) after surgery. No changes were made to the primary outcome variables following the commencement of the trial.

During surgery, anesthesia was delivered to all participants via inhalation induction with air/nitrous oxide and sevoflurane, intravenous supplementation with propofol and/or fentanyl 1-2 mcg/kg, anti-emetic prophylaxis with dexamethasone 150 mcg/kg and ondansetron 50 mcg/kg, acetaminophen suppository 40 mg/kg and morphine intravenous 100 mcg/kg.

Randomization to morphine or ibuprofen was achieved using a computer generated algorithm. The randomization algorithm was implemented by the research associate who assigned patients to either the morphine or ibuprofen group. The study clinicians and all care providers were blinded at the time of surgery. Parents were not blinded as they were required to fill their child's prescription. Parents were instructed to give the children acetaminophen (10-15 mg/kg per dose q4hours) and age-appropriate doses of morphine (0.2-0.5 mg/kg per dose q4hours) or ibuprofen (10 mg/kg per dose q6hours) as needed. Parents were instructed to start with a low dose (ie. 0.2 mg/kg) at first, and increase the dosage within the range (up to 0.5 mg/kg) at the next interval if needed based upon severity of pain. Upon discontinuation of morphine or ibuprofen, the monitors were returned to the study coordinators for data

extraction. For both groups, all adverse events were monitored and recorded by the parents. This included any signs of significant oral or nasal bleeding at which case they were instructed to return to the hospital emergency department for appropriate assessment and management. Pamphlets describing the signs of serious post-surgical bleeding requiring medical examination were provided to the parents. Secondary outcome variables included the rate of adverse drug reactions and tonsillar bleeding.

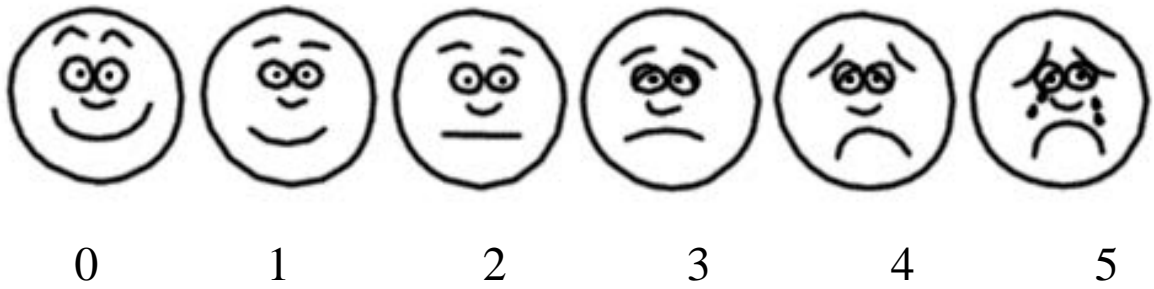
Analgesic effectiveness was assessed on post-operative Day 1 and Day 5 using the validated Objective Pain Scale (OPS) (37, 38) which was recorded by the study researchers in hospital, and then by the parents at home. Parents were trained by the research staff regarding completion of both pain scales. The OPS includes measurements of blood pressure, crying, movement, agitation and posture (Figure 4.1). Blood pressure measurements were performed only while the patient was in hospital. This validated tool has been extensively used to compare different analgesic modalities in children (39). In addition, on Day 1 and Day 5, the child completed a modified Faces Scale, a validated tool (ages 1-14) which is also commonly used for pain measurement, following adenotonsillectomy (Figure 4.2) (40). These pain scores as well as the number of days for a child to return to pre-operative diet, and the dosing schedule were returned to the research team for data analysis, following cessation of analgesia.

Pre and post tonsillectomy values were compared between the groups by Student's t test or Chi square test as appropriate.

Figure 4.1 The objective pain scale Post-operative blood pressure was collected in hospital by the research team and parents were instructed to monitor crying, movement, agitation and posture every 4-6 hours on postoperative Day 1 and Day 5 (38).

	Criteria	Points
Blood pressure	±10% preoperative value	0
	>20% preoperative value	1
	> 30% preoperative value	2
Crying	Not crying	0
	Crying but responds to loving care	1
	Crying and does not respond to loving care	2
Movement	None	0
	Restless	1
	Thrashing	2
Agitation	Asleep or calm	0
	Mild	1
	Hysterical	2
Posture	No special posture	0
	Flexing legs and thighs	1
	Holding hands to the neck	2

Figure 4.2 The modified Faces Scale (41) which parents were asked to complete with their child every 4-6 hours on postoperative Day 1 and Day 5.



4.5.3 Results:

From September 2012 to January 2014, a total of 91 children were consented to participate in this study (Figure 4.3). Demographic characteristics of the cohort can be seen in Table 4.1. In January 2014 we conducted an interim analysis, as planned in the original protocol, and the Drug Safety Monitoring Board was convened. In both groups, morphine or ibuprofen were used for a mean of 4 post-operative days (4.64 ± 1.87 days on ibuprofen, 4.04 ± 1.92 days on morphine). Acetaminophen was used for 4.46 ± 1.41 days by those randomized to receive ibuprofen and 4.86 ± 1.62 days in the morphine group. On the first post-operative night, only 13% of children receiving morphine exhibited improvement in the rate of desaturation events than before surgery, compared to 65% in those children receiving ibuprofen ($p < 0.01$) (Table 4.2). The number of desaturation events per hour (pre-operative to post-operative) was reduced by a mean of 1.79 ± 7.57 in the ibuprofen group compared to an average increase of 11.17 ± 15.02 in the morphine group ($p < 0.01$) (Table 4.2). The Hodges-Lehman effect size (difference between medians) was 8.1 with a 95% C.I of 6.4 to 14. One of the children randomized to the morphine group suffered from a severe adverse drug reaction related to oxygen desaturation, as described in Figure 4.4. There were no significant differences in the change in lowest oxygen saturation or the mean oxygen saturation following surgery between the two groups (Table 4.2). Tonsil size did not correlate with the change in desaturation event rates in either group ($R^2 = 0.005$, $p > 0.05$ ibuprofen, $R^2 = 0.036$, $p > 0.05$ morphine).

Mean modified Faces Scale scores on the first post-operative day were 2.76 ± 1.33 in the ibuprofen group (N=41) and 2.96 ± 1.02 in the morphine group (N= 45). On day 5 these pain scores decreased to 2.33 ± 1.19 and 2.02 ± 1.17 for the ibuprofen and morphine groups respectively. There were no differences in analgesic effectiveness as assessed by change in the modified Faces Scale score from Day 1 to Day 5 (0.21 ± 2.03 ibuprofen, 0.80 ± 1.41 morphine, $p = 0.29$). Regarding OPS, mean scores were 2.54 ± 2.17 in the ibuprofen group (N=41) and 2.05 ± 1.56 in the morphine group (N= 45) on post-operative Day 1. On day 5 these pain scores decreased to 2.29 ± 2.02 and 1.42 ± 1.52 for the ibuprofen and morphine groups respectively. There was no significant difference in the change in OPS score from Day 1 to Day 5 between the two groups (0.42 ± 1.42 ibuprofen, 0.40 ± 1.26 morphine, $p = 0.95$). The number of days to return to pre-operative diet was also not different between the two treatment groups (7.17 ± 5.23 days ibuprofen, 7.31 ± 3.82 days morphine, $p = 0.89$).

Tonsillar bleeding was reported in 3 children that received ibuprofen and 2 children that received morphine. One of the children who bled in the ibuprofen group required hospitalization, as did both of the children who bled in the morphine group. Adverse drug events were reported at similar rates by parents in the two groups including; sedation (ibuprofen 7% [2/30], morphine 15% [5/34]), constipation (ibuprofen 7% [2/30], morphine 9% [3/34]), nausea and vomiting (ibuprofen 13% [4/30], morphine 12% [4/34]), dizziness and confusion (ibuprofen 7% [2/30], morphine 21% [7/34]), refusing fluids/anorexia (ibuprofen 10% [3/30], morphine 3% [1/34]) and agitation (ibuprofen 3% [1/30], morphine 3% [1/34]). Night terrors were reported by 9% (3/34) of children receiving morphine. In the ibuprofen group 7% (2/30) reported fever and 3% (1/30) reported diarrhea.

As a result of the interim analysis, the Data Safety Monitoring Board instructed the research team to discontinue the study on January 31, 2014 and to inform the respective research ethics boards and Health Canada that there were significantly increased desaturations in the morphine group.

Figure 4.3. Recruitment flow diagram created using the CONSORT guidelines (42). Other reasons for approached patients not being randomized include: language barriers and parents asking specifically for morphine (not wanting to be randomized). Patients who did not follow randomization did not take the medication they were randomized to receive. All patients that were allocated to either morphine or ibuprofen were included in the intent to treat analysis. Only those with oximeter data available were included in the primary outcome analysis.

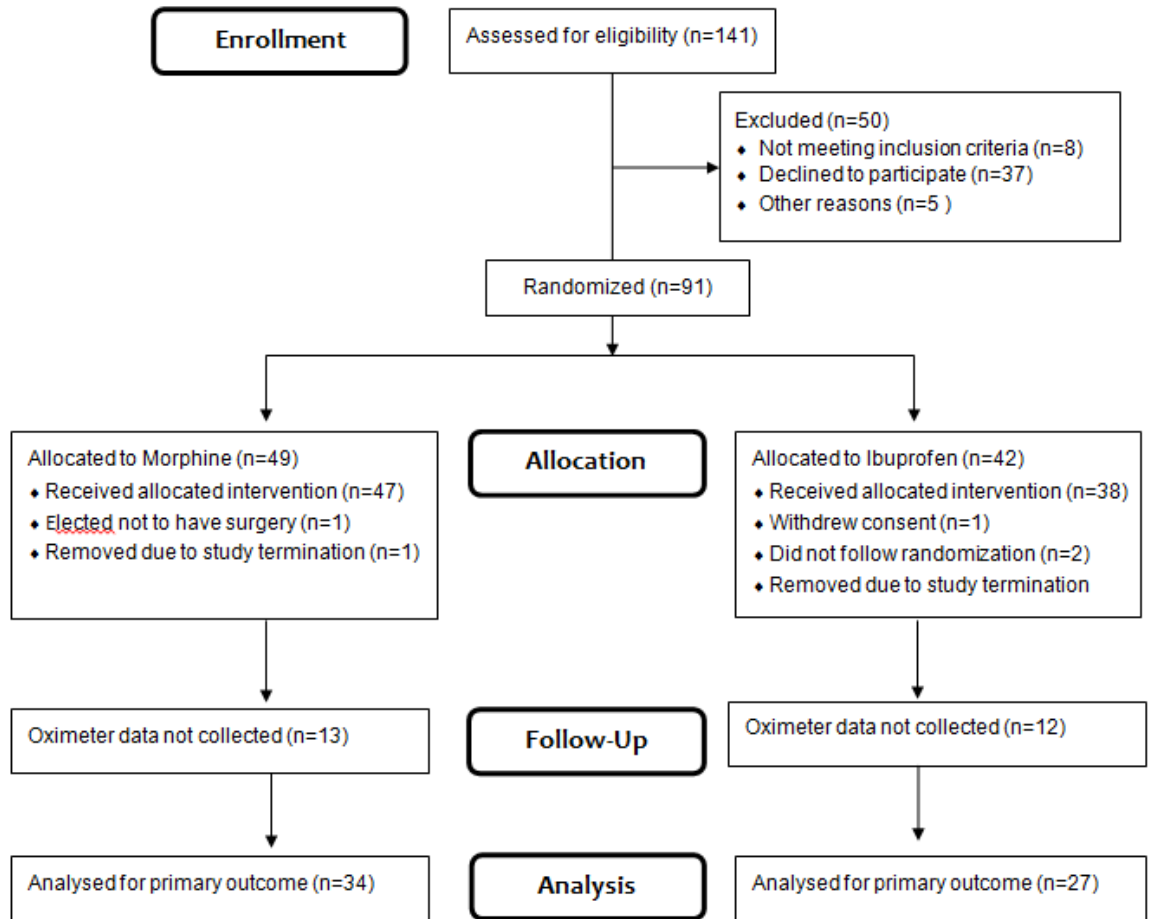


Figure 4.4 Serious adverse drug event details and timeline

A ten-year old Caucasian female (31kgs, BMI – 15 kg/m², Tonsil size = 4) returned to hospital the morning following her tonsillectomy (after three 6mg doses of morphine) due to prolonged vomiting, which the parents reported contained blood. In hospital she received 8 mg IV ondansetron, 445mg acetaminophen and 300mg ibuprofen. The emergency physician increased her morphine dose to 8mg q4hr prn. She was given IV morphine (3mg) in hospital and discharged that evening. Three hours following her 2AM morphine dose her parents noticed her lips were blue, she had a slow heart rate and was unresponsive and returned her to the emergency room. Her O₂ saturation upon arrival was 76% and she was promptly administered 0.05mg IV naloxone and supplemental oxygen. She was admitted into the PICU and morphine was discontinued. While admitted, chest x-rays revealed right lobe infiltrate, hyper-aeration and air space disease which was treated with 1500mg ceftriaxone. Following PICU discharge on post-operative day 4 she returned to hospital 5 days later with a viral upper respiratory tract infection. She has since made a successful recovery.

Day 1:

21:00 Patient arrived home from adenotonsillectomy, first dose of **morphine (6mg)**
Oximeter readings: Lowest O₂ sat 87%, mean O₂ sat 94.80%, 10.43 desats/ hour

Day 2:

2:30 **6mg morphine**, 15ml acetaminophen
5:00 patient begins vomiting, gravol suppository (5:22)
7:00 **6 mg morphine**, 15ml acetaminophen
10:00 brought to ER due to continued vomiting with blood
11:44 acetaminophen 445mg
13:59 ondansetron 8mg IV
14:40 **2mg IV morphine**
14:59 ibuprofen 300mg PO
16:31 **1 mg IV morphine**
18:10 **8 mg morphine PO** and discharge from hospital
22:00 **8mg morphine PO**, gravol suppository (21:00)

Day 3:

2:00 patient fever 103.3° F, given **8mg morphine PO**, 15 ml acetaminophen, gravol suppository (3:00)
5:00 patient not breathing, lips blue, heart rate slow, unresponsive, brought to ER
ER vitals temperature of 38, HR145, BP102/65, O₂sat 76%. Administered 0.05mg naloxone with supplemental O₂. Admitted to PICU, chest x-ray reveals right lobe infiltrate, hyper aeration and air space disease, administered 1500mg ceftriaxone

Day 4:

Patient discharged from PICU without long-term sequelae

Day 9:

Patient returns to hospital with a viral upper respiratory tract infection

Table 4.1. Patient demographics in both the morphine and ibuprofen group. Continuous variables are reported as mean \pm standard deviation and dichotomous variables are displayed as a percent value (N).

Demographics	Morphine (N = 46)	Ibuprofen (N = 38)
	Mean \pm SD Percent (N)	Mean \pm SD Percent (N)
Age (years)	5.07 \pm 2.45	5.14 \pm 2.25
Weight (kg)	27.36 \pm 8.78	22.38 \pm 9.59
BMI (kg/m ²)	17.31 \pm 3.00	18.29 \pm 4.56
Gender (female)	50% (23)	54% (22)
Pre-operative Tonsil Size	2.80 \pm 0.61	3.05 \pm 0.58
Diagnosis:		
Sleep Disordered Breathing (SDB)	57% (26)	48% (19)
Obstructive Sleep Apnea	32% (15)	45% (18)
SDB with recurrent tonsillitis	11% (5)	7% (3)
Ethnicity:		
Caucasian	87% (40)	93 % (38)
African American	7% (3)	7% (3)
Middle Eastern	4% (2)	0
South American	2 % (1)	0

Table 4.2. Primary outcome variables in the morphine and ibuprofen groups. Primary outcome variables in the morphine and ibuprofen groups are seen below. The number of children improved is defined as a child having fewer desaturation events per hour following surgery when compared to their preoperative values

	Ibuprofen (N = 26)	Morphine (N = 30)	P value
Lowest O₂ Saturation (%nadir)			
Pre-Op	85.39 ± 6.93	83.97 ± 7.86	
Post-Op	81.27 ± 15.81	81.63 ± 12.75	
Δ lowest O ₂ Saturation	3.96 ± 12.65	2.38 ± 12.30	0.64
Mean O₂ Saturation (% nadir)			
Pre-Op	97.41 ± 1.02	97.20 ± 1.22	
Post-Op	96.55 ± 2.07	95.00 ± 2.18	
Δ mean O ₂ Saturation	0.79 ± 2.33	2.13 ± 1.42	0.33
Total #desaturation events/hour			
Pre-Op	4.52 ± 7.87	3.64 ± 3.71	
Post-Op	3.04 ± 3.27	14.26 ± 11.85	
Δ total desaturation events/hour	- 1.79 ± 7.57	+ 11.17 ± 15.02	< 0.01
# children improved	65% (17/26)	13% (4/30)	< 0.01

4.5.6 Discussion:

Our study reveals that for children undergoing tonsillectomy, the standard dose of morphine analgesia increases the risk of oxygen desaturation, in comparison to ibuprofen. Tonsillectomy ± adenoidectomy have been reported to improve sleep disordered breathing in only 65% of children (14, 15). Perioperative respiratory complications necessitating a medical intervention may occur in over 10% of children undergoing adenotonsillectomy, with over 60% of these occurring in the immediate postoperative period (6, 43, 44). Desaturations can continue for an unpredictable period of time and in a substantial number of children, possibly due to prolonged changes in the threshold of response to hypoxemia. Moreover, children can experience laryngospasm, airway swelling and pulmonary edema, leading to further desaturation and even to respiratory arrest (45). In such cases, where apnea is not improved following surgery, providing opioids to relieve pain has the potential to increase respiratory depression, through the action of mu and kappa opioid receptor agonists decreasing respiratory drive and response to hypoxemia.

There are several factors contributing to the exaggerated respiratory effects of morphine and other opioids used post-tonsillectomy in children with sleep disordered breathing. Respiratory comorbidities including unresolved apnea, craniofacial disorders, bronchopneumonia, asthma, obesity, and respiratory tract infections combined with swelling following surgery, can further compound the respiratory effects of opioids. Hypercarbia is commonly seen in children with sleep disordered breathing resulting from an overall decrease in ventilation. This disturbance can be reversed following surgery, however the rate of

recovery and extent of reversal is not well understood. Residual respiratory acidosis, after tonsillectomy, may increase the delivery of nonionized morphine to the brain, due to an increase in cerebral blood flow and has the potential to increase respiratory compromise (46). In canine studies, hypercarbia has been shown to increase cerebral morphine concentrations, while serum morphine levels remained unchanged (47). Furthermore, morphine requirements for analgesia have been shown to be considerably variable in children, as much as 15 fold in some studies (48) occasionally necessitating large doses for adequate pain control which may lead to respiratory depression.

In this study, pain was managed with similar effectiveness by acetaminophen when in combination with either morphine or ibuprofen. A previous randomized trial assessing ibuprofen and acetaminophen with codeine, in 110 children following tonsillectomy, found no statistically significant difference in reported pain and satisfactory pain relief, as reported by parents at post-operative follow-up (49). The primary outcome measure in that study was tonsillar bleeding rates which, similar to our experience, was also not significantly different between the two groups. The hypothesis that morphine analgesia may eliminate respiratory complications by obviating the large variability seen in codeine metabolism and provide more predictable pharmacodynamic effect is rejected in the present study, showing similar unacceptable rates of postsurgical oxygen desaturations to codeine and hydrocodone (50).

Limitations of the present study include missing oximeter data due to children in both treatment groups refusing to sleep with the oximeter on their finger. Yet, the available sample

size provided sufficient statistical power to show the CNS-depressing risks of the opioid. As well, complete polysomnographic data was not obtained for our patients. While this may have provided more data, it may not have been well tolerated in the immediate post-operative period by children. Previous work has demonstrated that overnight oximetry correlates well with post-operative likelihood of adverse respiratory events, and that it is a useful modality in the preoperative evaluation of children with SDB prior to adenotonsillectomy (36, 51). Physiologic factors that have been previously identified as potential contributors to poor post-tonsillectomy outcomes include smaller tonsils, narrow epipharyngeal airspace and maxillary/mandibular protrusions (52). In this cohort, tonsil size did not correlate with an improvement in the number of desaturation events; airspace diameter and maxillary/mandibular orientation were not assessed. Finally, although parents were instructed to maintain hydration, fluid intake was not monitored in this cohort. This could potentially have affected pain scores as inadequate hydration has been shown to increase reported pain following tonsillectomy (53). The reported severe adverse drug reaction is confounded by the administration of intravenous morphine and the comorbid respiratory tract infection.

Pre-operative use of diclofenac and gabapentin (54) has been shown to decrease post-tonsillectomy opioid requirements children older than ten years of age, and the effectiveness of these medications in young children warrants further investigation. When compared to morphine, tramadol was shown to result in fewer respiratory events post-tonsillectomy however further work is required to assess the safety of tramadol as an alternative analgesic (55). Future studies should also address genetic variability in opioid response and analgesic

effectiveness by assessing both genotype and plasma drug levels to better characterize which children may be increasingly sensitive to post-tonsillectomy opioids.

In conclusion, the use of a standard morphine dose for post-surgical analgesia was associated with increased risk of oxygen desaturation. There were no differences seen in tonsillar bleeding events or in analgesic effectiveness. The results of this study support effective post-tonsillectomy analgesia in children using ibuprofen with acetaminophen.

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Chapter 5: Overall perspective in the context of future directions

Part of this chapter has been submitted for publication:

Kelly LE, Knoppert D, Roukema H, Rieder MJ, Koren G. Oral morphine weaning for neonatal abstinence syndrome at home vs. in hospital.

5.1 Preamble

As the number of women using opioids in pregnancy increases, so does the number of exposed neonates and the incidence of neonatal abstinence syndrome (NAS). In 2009, the reported hospital costs for the management of a newborn with NAS was \$53,400 compared to \$9,500 for a non-NAS related birth (1). During 2009 more than 13,000 infants were born in the United States who showed signs of NAS leading to an annual total hospital expenditure of over US\$720 million dollars (1). As the incidence of NAS continues to rise, developing safe and cost effective treatment strategies is more important than ever. The following chapter describes my proposed future research goals which aim to identify cost-effective management of this epidemic. It starts with a retrospective observational cohort study we recently completed and submitted for publication. This cohort study subsequently informs a proposal for a future randomized control trial.

5.2 Oral morphine weaning for neonatal abstinence syndrome in London, Ontario

Approximately 90% of drug abusing women are within the child bearing age range (15-39 years) (2, 3). According to the National Survey on Drug Use and Health, 4.4% of pregnant women reported using illicit substances within the past 30 days (4, 5). Opioids, including methadone and heroin are known to cross the placenta (6, 7) and *in utero* exposure can lead to neonatal withdrawal. Symptoms of NAS include various degrees of central nervous system effects such as high pitch cry, irritability, tremor and seizures as well as

gastrointestinal and metabolic disturbances (8). Currently, oral morphine is the most frequently used first line agent to treat NAS and in severe or unresponsive cases, phenobarbital or clonidine are used as adjuvant therapy (9-11). In order to ensure careful monitoring and treatment, neonates with suspected NAS are typically admitted to the neonatal intensive care unit (NICU). In the neonatal ward, the severity of NAS symptoms is commonly monitored by using the Finnegan Scale (9, 10, 12), and these scores dictate the initial morphine dose. Gradual tapering of morphine is typically done in hospital and can last from several days up to months (median of 30 days), which has a very high cost of hospitalization (3, 13).

In the United Kingdom, roughly 15% of neonatal hospital units reported discharging neonates with morphine into the community to be managed at home by the primary caregiver (9). This practice, however, has not been reported in North America. The objective of this study was to assess the safety and effectiveness of managing NAS at home with oral morphine weaning. Secondary outcome measures included an estimation of cost savings.

In London Ontario, two methods have been practiced side by side over the last few years:

- 1) Treating the baby throughout the entire NAS in hospital
- 2) Releasing the baby home to finalized treatment of NAS by the primary caretaker.

This has presented a rare opportunity to compare the safety, effectiveness and cost of these two treatment modalities. The primary research question is whether or not NAS treatment at home can be effective. Subsequent to this observational study I propose a randomised prospective study informed by the results of this pilot investigation.

5.3 Oral morphine weaning for neonatal abstinence syndrome at home vs. in hospital

5.3.1 Patients and methods:

This observational study included all neonates with NAS receiving oral morphine admitted between January 1, 2006 and December 31, 2010 at two academic health centers in London, Ontario, Canada. As per clinical routine, physicians at one institution kept most of its neonates in the NICU until morphine tapering was complete (“in hospital” group) while the those at the other center released stable neonates to go home with a weaning schedule (“at home” group). Neonates with NAS who were administered oral morphine were identified through pharmacy records and clinical databases. Anonymized data, from paper and electronic patient records included the number of days the neonate remained in hospital, oral morphine dosing, Finnegan Scale scores, and concomitant medication use. Demographic and clinical details were also collected. The number of hospital visits for further withdrawal treatment, emergency room visits, specialist referrals and outpatient/in-patient appointments were collected from electronic patient records for the first and second year of life. The number of in-patient appointments in the first year excluded the initial NAS treatment.

In both institutions, neonates were treated in accordance with the same NAS treatment protocol. Infants with suspected NAS were scored on the Finnegan Scale every 2 hours for the first 48 hours and every 4 hours thereafter. Scores reflect the infants’ activity over the previous four hours and morphine therapy was initiated for two scores greater than 8

or one score greater than 11. Morphine was started orally, unless an IV was already present in which case an initial morphine loading dose of 50µg/kg was given over at least 5 minutes and a continuous morphine infusion of 5 to 10µg/kg/hr (equivalent to 240-480µg/kg/day PO) was administered. If following morphine initiation the next score was above 8, the dose was increased by 2.5µg/kg/h. Medication doses in hospital were not changed for scores of six or seven. If the score fell below 5, the dose was reduced by 10% every 48 hours. If multiple drug exposure was suspected (e.g. sedatives, alcohol, barbiturates) or NAS was non-responsive to morphine, single doses of phenobarbital, clonidine or clonazepam were given. At one institution, neonates presenting as medically stable with persistently low Finnegan Scale scores (below 8) for a minimum of 24 hours were considered for discharge. If still on IV, neonates were first converted to oral morphine at a rate of 2x the intravenous dose.

Discharge home to complete the oral morphine taper was only considered if social stability was demonstrated, follow-up with a pediatrician was in place and caregivers were competent in administering the morphine doses. Specific dosing calendars were prepared by the pharmacy team for each individual neonate upon release home and a sample weaning calendar is seen in Figure 5.1. In all cases caregivers were educated regarding the symptoms of withdrawal and how to administer the weaning morphine doses. Caregivers were encouraged to return to the hospital with any concerns. Caregivers of children weaned in hospital were also assigned a social support case worker and a public health nurse. At both sites non-pharmacological interventions included minimizing lighting and stimulation, speaking softly, swaddling, and applying ointment cream (nystatin, zinc oxide) to the skin following frequent stools. Parents managing the morphine wean at home were encouraged to

continue these non-pharmacological interventions after discharge. This study was approved by the Research Ethics Board at Western University.

5.3.2 Results:

In a 4 year period 80 neonates were treated with oral morphine for NAS in the two institutions. There were 52 neonates who completed their oral morphine weaning at home, and 28 who remained in hospital until weaning was completed. Preterm births (34 to 36 weeks) occurred in 15% of all cases (12/80) and the mothers' median age was 26 years (17-41 years). Gestational age did not correlate with the length of stay in hospital ($R^2 = 0.0038$). In both groups most babies were delivered vaginally ("at home group", 63% [33/52], "in-hospital group", 71% [20/28]) without complications.

The majority of mothers (65%, 52/80) participated in a methadone maintenance program at the time of delivery. The most common reason for methadone use was addiction to slow release oxycodone (Oxycontin) (47%), followed by addiction to oxycodone plus acetaminophen (Percocet) (26%), heroin (23%) or morphine (4%). Cigarette smoking was reported by 82% of all mothers and no difference was seen between the groups. Demographic descriptors of both groups are shown in Table 5.1. Illicit drug use (marijuana, cocaine and/or opioids) was highly prevalent in both groups ("at home", 53% [24/45], "in hospital", 38% [10/26]) by womens' reports or positive urine screen. The Children's Aid Society (CAS), a public child welfare agency, placed 34% of all neonates into custody.

Clinical response variables are presented in Table 5.2. In neonates who completed oral morphine weaning “at home” the median number of days in hospital was significantly lower ($p = 0.042$) and fewer babies had to return to hospital for withdrawal treatment ($p = 0.044$) compared to those who completed their wean “in hospital”. The neonates who continued morphine at home following hospital discharge remained in hospital for a median of 6 less days than those who weaned in hospital. Neonates weaned “at home” were on oral morphine for significantly more days ($p < 0.001$) and were significantly more likely to have had phenobarbital, clonidine or clonazepam while in the NICU ($p = 0.003$). Although there was no difference in the number of NAS scores above 8, neonates weaned at home were significantly more likely to receive an adjuvant therapy in the NICU, indicative of either more severe withdrawal or differences in group practice. Breastfeeding data were available for 61 mother infant pairs, 40% of which initiated breastfeeding while in hospital (Table 5.1).

Breast fed neonates had significantly fewer NAS scores above 8 (median 10 range [0-77]) vs. no breastfeeding (16 [1-85]), $p = 0.02$. The number of emergency room visits, in/out patient appointments and specialist referrals in the first and second year of life are displayed in Table 5.3. There was one case of sudden unexpected infant death syndrome in the cohort of children weaned at home attributed to bed sharing and the presence of an unsafe sleeping environment.

Figure 5.1. Example of an oral morphine weaning calendar provided to caregivers. To protect confidentiality the names and contact number have been changed.

JUNE 2009

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 150mcg (0.15mL) every 6 hr	2 150mcg (0.15mL) every 6 hr	3 100mcg (0.1mL) every 6 hr	4 100mcg (0.1mL) every 6 hr	5 50mcg (0.05mL) every 6 hr	6 50mcg (0.05mL) every 6 hr
7 50mcg (0.05mL) every <u>8hr</u>	8 50mcg (0.05mL) every <u>8hr</u>	9 50mcg (0.05ml) every <u>12hr</u>	10 50mcg (0.05ml) every <u>12hr</u>	11 50mcg (0.05ml) every <u>24hr</u>	12 50mcg (0.05ml) every <u>24hr</u>	13 <u>STOP</u>
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	Keep doctor informed of Jane's progress	Prepared by John Smith, Pharmacy Services (555) 555-5555 ext 5555		

Table 5.1. Demographic characteristics of neonates treated for neonatal abstinence syndrome (NAS) with oral morphine at-home and those who remained in-hospital until the morphine taper was complete. Data are presented as median (range) or percent of total. The Mann Whitney U test was used to determine significance for continuous variables and the Fischer Exact test for the categorical data. The level of statistical significance was set at 0.05 where NS is not significant.

	At-home Weaning (N = 52)	Hospital Weaning (N = 28)	P Value
Maternal Age	26 (17-36)	25 (18-41)	NS
Premature (< 37 weeks)	11% (6/52)	21% (6/28)	NS
Low Birth Weight (< 2500g)	13% (7/52)	18% (5/28)	NS
Smoking	79% (30/38)	88% (14/16)	NS
Cocaine	34% (10/29)	21% (3/14)	NS
Marijuana	41% (12/29)	29% (4/14)	NS
Benzodiazepines	21% (6/29)	29% (4/14)	NS
Antidepressants	21% (6/29)	29% (4/14)	NS
Initiated breastfeeding in hospital	41% (17/41)	33% (7/21)	NS
Father involved in care	72% (28/39)	75% (15/20)	NS
Received appropriate prenatal care	25% (7/28)	47% (9/19)	NS
Some/minimal prenatal care	35% (10/28)	11% (2/19)	NS
Did NOT receive any prenatal care	40% (11/28)	42% (8/19)	NS
CAS Apprehensions	31% (16/52)	39% (11/28)	NS

Table 5.2. Clinical response of neonates treated with weaning doses of oral morphine at-home and those who remained in-hospital until the morphine taper was complete. Data are presented as median (range) or percent of total. The Mann Whitney U test was used to determine significance for continuous variables and the Fischer Exact test for the categorical data. The level of statistical significance was set at 0.05.

	At-home Weaning (N = 52)	Hospital Weaning (N = 28)	P Value
Number of days in NICU	16 (3-54)	22 (7-51)	0.04
Number of times NAS score was over 8	12.5 (0-132)	10 (0-71)	0.13
Number of mothers on methadone	65% (34/52)	64% (18/28)	1.00
Methadone dose (mg/day)	80 (20-115)	80 (25-130)	0.77
Babies receiving adjuvant therapy*	31% (16/52)	4% (1/28)	< 0.01
Return to hospital for withdrawal treatment	2% (1/52)	14% (4/28)	0.04
Total number of days on oral morphine	32 (12-117)	19 (6-48)	< 0.01

*Adjuvant therapy included phenobarbital, clonidine or clonazepam given in the NICU.

Table 5.3. The median number of emergency room visits, specialist referrals and in/out patient appointments from birth until September 1, 2013 required by neonates weaned at-home and those who completed their morphine taper in hospital. The number and type of specialist referrals is also displayed. Year one represents the first twelve months of the child's life and year two represents months twelve to twenty four.

	At-home Weaning (N = 52)	Hospital Weaning (N = 28)
Number of times, in-patient*, Year One	0 (0-3)	0 (0-3)
Number of times, in-patient, Year Two	0 (0-1)	0 (0-2)
Number of out-patient appointments in Year One	0 (0-21)	0 (0-31)
Number of out-patient appointments in Year Two	0 (0-8)	0 (0-11)
Number of visits to ER in Year One	1 (0-8)	1 (0-6)
Number of visits to ER in Year Two	1 (0-4)	0 (0-6)
Percent of children who were referred to at least one specialist	46% (24/52)	36% (10/28)
Specialist Referrals (N):		
Allergy/Immunology	2	0
Cardiology	3	1
Developmental Follow Up	5	3
Endocrinology	1	0
Gastroenterology	1	0
Genetics	2	1
Haematology	2	1
Nephrology	2	1
Neurology	3	0
Ophthalmology	4	1
Otolaryngology	3	2
Physiotherapy	1	2
Respirology	1	1
Speech Language Pathology	3	0
Surgery	4	1
Urology	3	1

*Initial NAS treatment was excluded.

5.3.3 Discussion:

To the best of our knowledge, this is the first study to describe a cohort of neonates with NAS who completed their oral morphine weaning outside of a hospital setting. In neonates who were weaned at home we did not detect an increased risk for emergency room visits or in/out- patient appointments. The rate of return to hospital for further withdrawal management was significantly lower in those infants weaned at home as compared to those who remained in hospital, suggesting that a slower tapered wean may actually be advantageous in managing NAS. At home oral morphine weaning offers several advantages including a slower morphine wean, increased mother-infant bonding time and a decrease in hospital costs. The average daily cost of a bed in the NICU in London, Ontario in 2013 is estimated at \$1800.00/night (personal communication). Sending neonates home to finish their morphine wean is associated with a median of 6 fewer days in hospital and provided a potential cost saving of approximately \$560,200.00. To more accurately estimate the amount of savings the more common need for re-hospitalization of neonates treated “in hospital” must be included. There were no significant differences in the total number of emergency room visits, specialist referrals, or in-/outpatient appointments between those weaned at home and in hospital. As the number of infants exposed to opioids with subsequent withdrawal symptoms increases globally and rapidly rising health costs it is urgent to assess safe and cost-effective treatment options.

Our study documents that completing morphine weaning at home may take more time than a completed in hospital wean. The fact that significantly more in hospital babies needed

to be re-hospitalized suggests that the weaning in the hospital was often too aggressive. There was one fatality in the group of neonates on morphine weaned at home. A previous study by our group suggested that there is no increased risk for mortality under the age of one year among infants exposed to methadone *in utero* (14). Further analysis outside the timeframe of this cohort revealed a second fatality attributed to SUDI in 2005 of a child whose wean was completed in hospital. A summary of these two fatalities has been previously described (12). There is therefore limited evidence to suggest that at home oral morphine weaning increased the risk for SUDI.

In our cohort reported benzodiazepine use (33%) was lower than previous reported rates of 50%; however, cocaine and marijuana incidence are similar (9). Studies suggest that cocaine increases the severity and frequency of NAS symptoms in those abusing opioids in pregnancy (15). Furthermore, benzodiazepine use is known to increase the length of NAS treatment as symptoms of benzodiazepine exposure often confound NAS (3). Concomitant use of cocaine and/or benzodiazepines was similar between groups and therefore is not a confounding factor for determining length of stay in our cohort. Breastfeeding was significantly associated with a less severe opioid withdrawal course, a finding that has been confirmed with other cohorts (16). As well as providing optimal nutrition, breast milk can provide small amounts of maternal opioid to ease withdrawal. Breastfeeding is recommended and should be encouraged for HIV-free patients on a methadone maintenance program whether weaned in hospital or at home.

This study has several limitations that require acknowledgement. This was not a randomized trial and hence uncontrolled bias could affect the results. The fact that the characteristics of the mothers and their opioid use did not differ significantly between the groups suggests that such bias, if it exists, is not major. Secondly, our study lacks follow-up. Many substance abusing mothers do not have permanent addresses or cellular phones, which made follow-up impossible. Furthermore, due to the retrospective nature of this study, we were unable to obtain all relevant data, such as child nutrition and social environment. A further limitation includes the data source for the number of emergency room visits, in-patient appointments and hospital visits which were assessed using electronic patient records and did not include general pediatrician care or specialist appointments outside of the hospital networks. The number of outpatient appointments included pediatrician appointments at both hospital sites. To address these limitations, our findings should be corroborated by a prospective, randomized trial before validated recommendations regarding at home oral morphine weaning can be made.

In summary, this observational study assessed the safety of neonatal oral morphine weaning at home versus in hospital for the management of NAS following *in utero* opioid exposure. A significantly lower return to hospital rate for further withdrawal treatment was identified in neonates weaned at home. These data suggest that training caregivers and sending neonates home with an oral morphine weaning calendar may present a safe and more cost effective measure for treating neonatal abstinence syndrome (vs in hospital).

5.4 Future Direction: Investigating the safety of at-home oral morphine weaning and pharmacogenomic predictors of neonatal abstinence syndrome

5.4.1 Rationale

Our retrospective observational study suggested that oral morphine weaning at home was as safe as weaning in hospital. Furthermore by extending the length of the morphine wean, and providing a slower tapering-off of morphine, neonates were significantly less likely to return to hospital for withdrawal management. London, Ontario remains one of the only sites in Canada to send neonates home with oral morphine weaning calendars. Before recommendations can be made at the National level, the findings from our observational cohort study require verification in a randomized prospective clinical trial. In order to assess the effectiveness of at home oral morphine weaning, the primary outcome variable to be investigated will be the return to hospital following discharge for withdrawal treatment.

The previously discussed literature supports an effect of genetic variability in predicting adult and pediatric opioid response; however the pharmacogenomic implications of *in utero* opioid exposure on the neonate remain unclear. Results from our pilot study (Chapter 2) suggest that CYP2B6 may play an important role in clearing the toxic S-methadone metabolite and that SNPs in P-glycoprotein may affect the rate of placental opioid transfer. This pilot study confirmed the feasibility of recruiting patients through participating family physician clinics including the collection of neonatal blood to be sampled for genotyping and

evaluation of plasma drug concentrations. A previous report by Wachman et al. (17) associated neonatal polymorphisms in COMT and OPRM1 with the severity of NAS following *in utero* opioid exposure however our pilot study did not have a sample size sufficiently large to corroborate these findings. These polymorphisms, as well as others known to affect pediatric opioid response should be assessed.

5.4.2 Hypothesis and research aims

Continued oral morphine weaning following hospital discharge is as safe and effective as weaning in hospital. Furthermore, we hypothesize that genetic and clinical factors will predict the severity of NAS.

The aims of this project include:

- 1) To investigate the safety and effectiveness of at-home oral morphine weaning in a prospective randomized clinical trial
- 2) To investigate the impact of maternal pharmacogenomics on neonatal opioid levels
- 3) Evaluate maternal pharmacogenomics as a predictor of neonatal abstinence syndrome severity
- 4) To investigate the impact of neonatal pharmacogenomics on neonatal opioid levels
- 5) To evaluate neonatal pharmacogenomics as a predictor of the severity of neonatal abstinence syndrome
- 6) To examine the role of previously identified clinical risk factors (breastfeeding, maternal methadone dose, prematurity, polysubstance use) with regard to severity of neonatal abstinence syndrome in a prospective clinical trial

5.4.3 Future study design

This multicentre clinical trial will recruit patients from Victoria Hospital in London, Ontario and St. Joseph's Hospital in Toronto, Ontario. This prospective clinical trial will randomize neonates showing stable Finnegan scale scores (4 consecutive scores below 8) with demonstrated social stability to complete their morphine wean in-hospital or to continue their wean post-discharge. Pharmacogenomic analysis will include the following genes: CYP2B6, CYP3A4, OPRM1, ABCB1 and COMT.

5.4.3.1 Inclusion and exclusion criteria

Inclusion criteria:

1. The mother has taken methadone for a minimum of three months during pregnancy
2. Mother abstains from use of illicit substances (as assayed in urine)
3. Caregiver demonstrates social stability with a secure home environment and support system in place, including a contact telephone number and address
4. Neonate has had consecutively low (below 8) Finnegan scale scores that have not increased in 24hours
5. Morphine wean has been switched from IV to PO if necessary
6. Neonate has no other medical condition requiring hospitalization
7. Proper health care follow-up is in place including; social worker, public health nurse, community services, and/or pediatrician

Exclusion criteria:

1. Neonates born with major congenital abnormalities or any condition that is likely to extend the need for hospitalization
2. Delivery complications, other than NAS, requiring administration of opioids
3. Lack of informed consent

5.4.3.2. Methodology

Following approval from the Research Ethics Board at The University of Western Ontario and St. Joseph's Hospital, informed consent will be received by the research team. The research team will include neonatologists, pediatricians, lactation consultants, public health nurses and social services. Clinical and demographic data will be collected from each patient using the data collection sheet (Figure 5.2). Once a neonate has stabilized NAS scores and if necessary their wean has been converted from IV to PO, they will be randomized to continue PO wean in-hospital or will be instructed on how to administer oral morphine according to an oral morphine weaning calendar prepared by the study pharmacist. All patients at both sites will complete the demographic data collection sheet. Cord blood samples will be collected to measure methadone concentration, and to extract DNA to genotype for genetic polymorphisms in CYP2B6, p-glycoprotein, COMT, and OPRM1. An additional 500µl blood sample will be collected at approximately 24 hours of life to measure serum methadone/EDDP concentrations. This sample will be taken in conjunction with the Ontario Newborn Screening standardized collection protocol. Methadone concentrations and genotyping will be completed as previously described in the pilot study. The research staff will contact the primary care giver at 3months of age to collect data regarding neonatal

wellbeing and to address any further health concerns. Hospital electronic patient records will be used to collect information on any further medical appointments and emergency room visits. At the end of a 6month period, data regarding return to hospital for further withdrawal management will be collected from electronic patient records and anonymously entered into the study database.

5.4.3.3 Sample size calculation

Previous data indicate that 14% of children who are weaned in-hospital return to hospital following discharge for further withdrawal management, compared to only 2% of neonates weaned at home. These data suggest that a slower oral morphine wean at-home (in cases where social stability can be demonstrated) is as effective in managing NAS, compared to completing the full wean in hospital. In order to compare two independent samples using the inference of proportions previously described and a power of 0.80, $\alpha = 0.05$, 80 neonates are needed in each arm.

5.4.3.4 Strengths and limitations

Strengths of this study include minimal invasiveness as the blood sample will be collected in conjunction with standard clinical tests. By recruiting at two centres, this study will allow for increased recruitment and sufficient variability to establish genetic associations. This study will be the first to prospectively assess the effectiveness of at-home oral morphine weaning and is likely to impact on future NAS management. The benefits of at home oral morphine weaning include an increase in bonding time for mother and baby as well as a decrease in overall hospitalization cost. Limitations include not collecting blood samples at

multiple time points which restricts the potential for pharmacokinetic analysis. While every attempt will be made to follow neonates for a 3 month period, some prospective data may be unattainable due to unreachable patients. Researchers hope that by pre-emptively including the entire circle of care including pediatricians, nurses and social services this will greatly improve the collection of follow-up data, identified as a major limitation in the earlier pilot study.

Figure 5.2. Sample of information gathering sheet to be completed with all patients at both sites.

Study ID number: _____

D.O.B: _____

Date of Hospital Discharge: _____

Hospital:

- St. Joseph's, Toronto
- Victoria, London

Neonatal Demographics			
Gestational Age (weeks):			
Birth weight (grams):			
Length (cm):			
Head Circumference (cm):			
Gender:			
APGAR SCORES	1 MIN	3 MIN	10 MIN
Delivery:			
<input type="checkbox"/> Vaginal <input type="checkbox"/> C/S or episiotomy			
Delivery complications: Y/N			
Explain:			
Maternal Demographics:			
Maternal age (years):			
Gravidity (TPAL):			
Prenatal care Y/N			
Education/Occupation:			
Maternal Prescription Drugs Y/N			
List:			
Father is involved in care of baby Y/N			
Maternal Opioid Use:			
Methadone Dose at delivery:			
Methadone Duration:			
Reason for use:			
Maternal smoking status Y/N			
Opioid use other than methadone Y/N			
List:			
Alcohol Y/N			
Cocaine Y/N			
Marijuana Y/N			

5.5 Overarching conclusions

In 2010, Canada surpassed the United States with the highest prescription opioid consumption per capita and since 2001 opioid poisonings in children have increased by 22% (17). Currently opioids are the greatest cause of pediatric deaths attributed to pharmaceutical poisoning, resulting from accidental overdose and therapeutic error (17). As opioid use increases in the adult population, so does exposure of neonates and infants to opioids through pregnancy and in breast milk as well as accidental poisoning due to an increase in availability in the home. Infants and children are prescribed opioids for analgesia and the high rate of interindividual variability in pharmacokinetics makes dosing challenging. Determining doses of opioids in young children is especially challenging as the majority of pharmacokinetic data have been obtained in adults. Furthermore, pain is a difficult sensation to quantify, especially in very young children who are unable to vocalize changes in their level of pain. Pain perception is influenced by a combination of inputs from memory, limbic systems, stress mechanisms and sensory signalling (18). This means that the sensation of pain can vary not only between individuals but also depending on one's attention, mood, memories, making analgesic effectiveness difficult to quantify and highly variable (18). In order to safely manage pediatric pain with opioids it is important to identify clinical and genetic risk factors that can aid in predicting how a child will respond to a standard dose of drug. The recent discovery of morphine uptake via the hepatic OCT1 transporter has been shown by one group to affect the pharmacokinetics of morphine response in children. This finding should be corroborated in other cohorts and investigated further in adults. The impact of genetic polymorphisms on opioid response is multi-factorial. Future research regarding clinical

models of predicting opioid response in children must consider new genetic markers, such as OCT1, while also accounting for clinical sources of variability.

Opioids are among the world's oldest known drug class and were not subjected to the pharmacokinetic/pharmacodynamic testing required to bring a drug on to today's market. Investigating clinical and genetic factors has shed light on some of the variability seen in pediatric opioid response. In an era where bedside pharmacogenomic testing is within reach the importance of prospective clinical evidence is greater than ever. In the case of codeine exposure in breast milk we showed that by controlling clinical risk factors we could mitigate the influence of genetics on determining codeine toxicity in the breastfed neonate (Chapter 3). Genetic factors were implicated in several fatalities associated with codeine exposure post-tonsillectomy. These case reports, along with the supporting literature, led the FDA to contraindicate codeine in this population. Following tonsillectomies improvement in respiratory parameters can take several days and prescribing an opioid has the potential to increase respiratory complications in some children (19). Further investigation, including a randomized clinical trial (Chapter 4) suggested that opioids may be responsible for increasing the rate of desaturation events per night following tonsillectomy in children with sleep disordered breathing. Future studies should address the safety and effectiveness of lower morphine doses or alternating morphine and ibuprofen doses in these children.

In the adult population, methadone response is associated with several genetic polymorphisms (20-22). The effects of these polymorphisms on the severity of NAS in the

newborn are currently unclear and require further investigation. The use of genetic factors to predict NAS may be beneficial in initiating early non-pharmacological management for those at an increased risk or pre-emptive therapeutic management.

While the use of opioids to manage pain in pediatric medicine is not likely to subside, dynamic growth in the field of opioid pharmacogenomics over the last two decades has allowed us to understand some of the variability seen in opioid safety and effectiveness. The increasing availability and decreasing cost of genome wide sequencing is likely to allow us to elucidate additional rare variants or new genetic associations which will enhance our understanding of the complexities governing variability in the perception of pain in children, as well as their response to analgesics. In conclusion, current data support a role for both clinical and genetic factors in determining pediatric opioid response. Based on our current knowledge dosing decisions for pediatric opioid use must be made on a case-by-case basis as standard dosing is not safe in all children.

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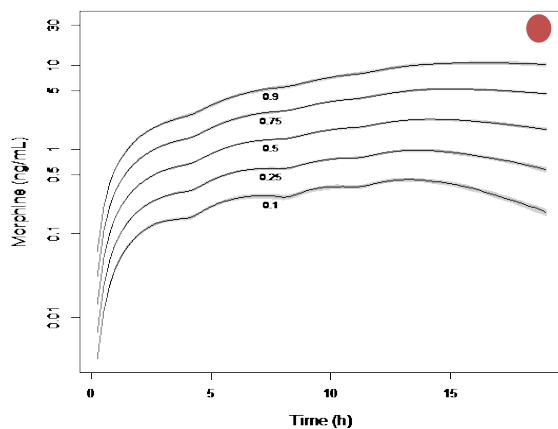
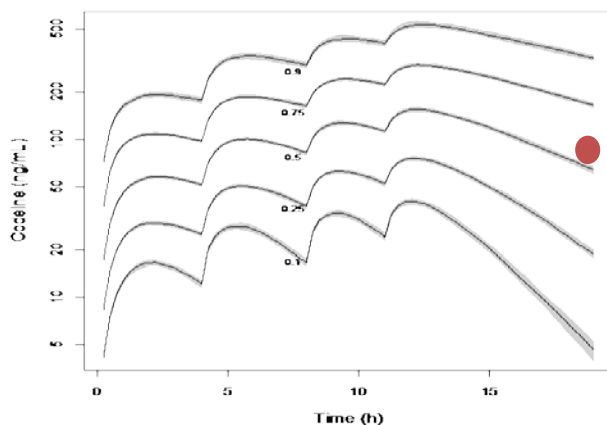
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PubMed PMID: 21902500.

Appendices:

Appendix 1: A pharmacokinetic model using Pmetrics software (Los Angeles, United States).

Model was constructed based on published pediatric pharmacokinetic characteristics to simulate expected time concentration profiles for codeine and morphine based on age, weight, and dosing schedule. Red dot indicates the patient in case 3 with measured concentrations within the 50th percentile for codeine and 99th percentile for morphine suggestive of a functional gene duplication



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Licensed content author	Lauren E. Kelly, Michael Rieder, John van den Anker, Becky Malkin, Colin Ross, Michael N. Neely, Bruce Carleton, Michael R. Hayden, Parvaz Madadi, Gideon Koren
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Appendix 3: Research Agreement between UWP and the Ministry of Community Safety and Correctional Services, Office of the Chief Coroner, Ontario Forensic Pathology Services and the Centre of Forensic Sciences

RESEARCH AGREEMENT FREEDOM OF INFORMATION AND PROTECTION OF PRIVACY

THIS AGREEMENT is made between:

HER MAJESTY THE QUEEN IN RIGHT OF THE PROVINCE OF ONTARIO
as represented by

**The Ministry of Community Safety and Correctional Services
Office of the Chief Coroner (OCC), Ontario Forensic Pathology Services (OFPS)
And The Centre of Forensic Sciences (CFS)**
(hereinafter referred to as the "Institution")

- and -

The University of Western Ontario
(hereinafter known as "UWO")

- and -

Dr. Gideon Koren (principle investigator)
Lauren Kelly (Coordinator)
(hereinafter referred to as the "Researcher")

WHEREAS Dr. Gideon Koren is the Director of the MotherRisk Program, Professor of Pediatrics, Pharmacology, Pharmacy and Medical Genetics, The University of Toronto, Professor of Medicine, Pediatrics and Physiology/Pharmacology and the Ivey Chair in Molecular Toxicology University of Western Ontario and the Principal Investigator. Lauren Kelly is PhD. Candidate, Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, University of Western Ontario and is the Coordinator and will be doing data collection and analysis.

AND WHEREAS the Researcher has indicated interest in researching all files relating to infants who received neonatal abstinence treatment due to the mother's prenatal methadone use;

AND WHEREAS the Researcher is interested in researching the incidence of such effects of eight completed files provided by the Office of the Chief Coroner

AND WHEREAS the Researcher has indicated that the purpose of his/her research is to identify risk factors and to determine the safety and efficacy of the two approaches to babies who require a long time to wean from oral morphine.

NOW THEREFORE the Researcher understands and promises to abide by the following terms and conditions:

1. The Researcher acknowledges that he/she will have access only to the completed closed or Q'ad records or files provided to them by the Office of the Chief Coroner (OCC), as a branch of the Institution.
2. The Researcher undertakes not to seek or obtain copies of material or files made available to him/her by the Institution.
3. The Researcher will not use the information in the records for any purpose other than the RFP approval unless the Researcher has the Institution's written authorization to do so: Collating non-nominal data (file numbers for which there are no names to link the information to individuals)
4. The Institution's representative is the *Freedom of Information and Protection of Privacy* Coordinator (the "Coordinator"). The Institution may designate a different representative by written notice to the Researcher at any time.
5. The Researcher shall be responsible for Project Management.
6. The Researcher undertakes not to give access to or to use or to disclose personal information, in a form in which the individual to whom it relates can be identified, to anyone except to such individuals as may be approved, in writing, by the Institution from time to time.
7. Before disclosing personal information to persons the Researcher shall enter into an agreement with those persons to ensure that they will not disclose it to any other person. Any such agreement shall be approved with respect to form and content by the Institution before being signed by such other person. The Researcher shall provide the Institution with executed copies of all such agreements.
8. The Researcher agrees that the Institution may, in its sole discretion, carry out security checks on such individuals who will have access to any personal information covered by this Agreement.
9. The Researcher shall hold the data in secure storage. The Researcher shall destroy all individual identifiers in the information once the project is completed. The Researcher shall notify the Institution in writing upon the destruction of personal identifiers in the information obtained from the Institution.

10. The Researcher shall provide the Institution with interim reports if any. In addition, the Researcher shall provide the Institution with a copy of a draft final report, if any, prior to the finalization of the report.
11. The Branch of the Institution shall be provided with an opportunity to review the draft final report or manuscript, if any, and may, in its own discretion, make representations as to its contents prior to the completion of the final report or manuscript.
12. The Researcher shall discuss with the principal investigator the involvement of the members of the OCC or Ontario Forensic Pathology Services (OFPS) staff in a manner in keeping with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research; Authorship and Contributorship (http://www.icmje.org/ethical_1author.html)

The nature of the OCC/OFPS staff members' participation as an author (or not) should be mutually agreed upon before the research is commenced. In certain research, the role may evolve, in which discussions regarding authorship should occur at the earliest time this evolution is recognized.
13. Upon review and discussion of the draft final report, if any, by the Institution, the Researcher shall consider the representations of the Institution prior to finalizing the final report. **The Researcher shall provide a final report to the Institution upon completion and forward a copy of all publications arising from data obtain from the Office of the Chief Coroner.**
14. The Researcher shall keep the information in a physically secure location to which access is given only to the Researcher. The Institution reserves the right to inspect such location, at any time. The Researcher consents to inspection by the Institution at any time of the location where the information is stored.
15. The Researcher shall not contact any individual to whom personal information relates, or the next of kin of such individual, directly or indirectly, without the prior written authority of the Institution.
16. The Researcher agrees to pay all costs incurred by the Institution under this Agreement.
17. The Researcher agrees that any method of secure transportation of the data is subject to the approval of the Institution.

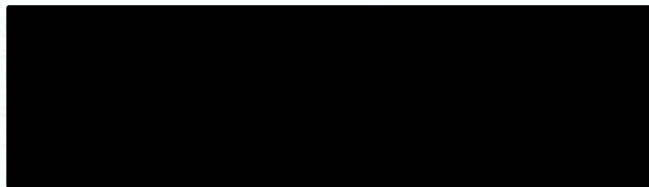
18. The Researcher agrees to indemnify the Institution, its employees and agents, against all costs, losses, expenses and liabilities incurred as a result of a claim or proceedings related to this Agreement, unless it was caused by the negligence or willful act of an employee of the Institution while acting within the scope of his or her employment.
19. The Researcher agrees to abide by the provisions of Copyright law in the performance of their obligations under this Agreement.

The Researcher further agrees to indemnify the Institution, its employees and agents, against all costs, losses, expenses and liabilities incurred as a result of a claim or proceeding resulting from a breach of Copyright law.

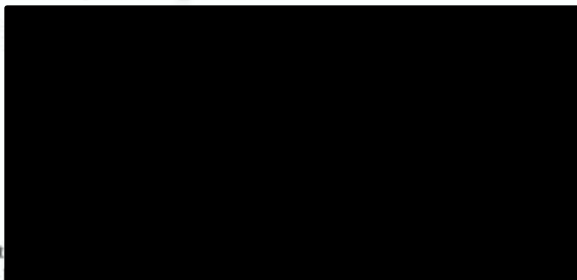
20. Notices under this Agreement shall be in writing and sent by personal delivery, or by ordinary prepaid mail.

Notices to the parties shall be sent to the following addresses:

Institution:
(Branch)



Principle Investigator:



Contact
Email:

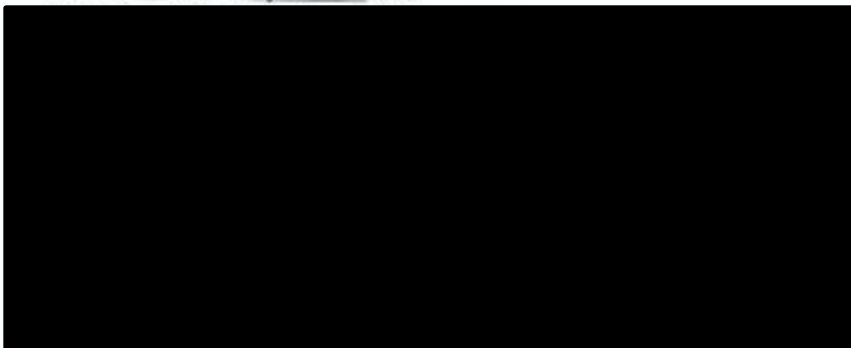
21. The parties may designate in writing to each other a change of address at any time.
22. The Researcher shall notify the Institution in writing immediately upon becoming aware that any of the conditions set out in this agreement have been breached.

23. This Agreement and the rights, obligations and relations of the parties shall be governed by and construed in accordance with the laws of the Province of Ontario and in particular the Freedom of Information and Protection of Privacy Act and the federal laws of Canada applicable herein. The parties do hereby attorn to the jurisdiction of the Courts of the Province of Ontario.
24. Under section 61 of the Freedom of Information and Protection of Privacy Act, any person who wilfully discloses personal information in contravention of the Act or who wilfully maintains a personal information bank that contravenes the Act or who makes a request under the Act for access to or correction of personal information under false pretenses is guilty of an offense and on conviction is liable to a fine not exceeding Five Thousand Dollars (\$5,000).

Acknowledgement

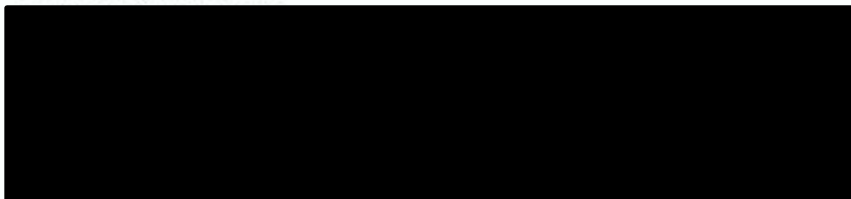
Having read this Agreement, I hereby agree to act in accordance with all terms and conditions herein.

SIGNED 8 day of April 2011

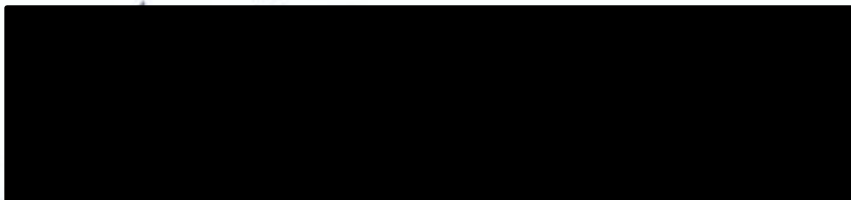


Ministry of Community Safety and Correctional Services

Office of the Chief Coroner

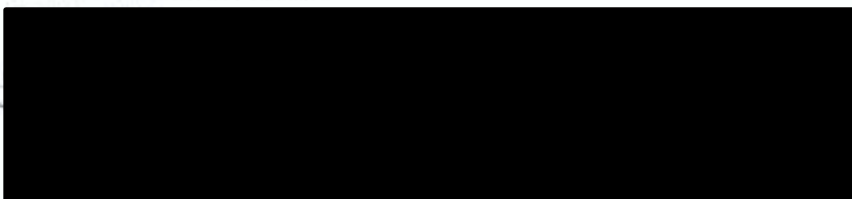


Ontario Forensic Pathology Service



Ontario Forensic Pathologist of Ontario

The Centre of Forensic Sciences



Appendix 4: Ethics approvals for methadone levels and pharmacogenomics pilot study



Office of Research Ethics

The University of Western Ontario
 Room 4180 Support Services Building, London, ON, Canada N6A 5C1
 Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
 Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D. Knoppert

Review Number: 15915E

Review Level: Expedited

Review Date: February 11, 2009

Protocol Title: The pharmacokinetics and acute effects of in utero methadone exposure on newborns

Department and Institution: Pharmacy, St. Joseph's Health Care London

Sponsor:

Ethics Approval Date: March 18, 2009

Expiry Date: September 30, 2009

Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent, Telephone Script.

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

**LAWSON HEALTH RESEARCH INSTITUTE
CLINICAL RESEARCH IMPACT COMMITTEE**

RESEARCH OFFICE REVIEW NO.: R-09-094

PROJECT TITLE: The pharmacokinetics and acute effects of in utero methadone exposure on newborns

PRINCIPAL INVESTIGATOR: Dr. D Knoppert

DATE OF REVIEW BY CRIC: March 23, 2009

Health Sciences REB#: 15915E

Please be advised that the above project was reviewed by the Clinical Research Impact Committee and the project

Was Approved

**PLEASE INFORM THE APPROPRIATE NURSING UNITS,
LABORATORIES, ETC. BEFORE STARTING THIS
PROTOCOL. THE RESEARCH OFFICE NUMBER MUST BE
USED WHEN COMMUNICATING WITH THESE AREAS.**

Dr. David Hill
V.P. Research
Lawson Health Research Institute

All future correspondence concerning this study should include the Research Office Review Number and should be directed to Sherry ~~Boice~~, Room C210, Nurses Residence, South Street Campus.

cc: Administration

Appendix 5: Ethics approval for codeine in breastfeeding



Office of Research Ethics

The University of Western Ontario
 Room 4180 Support Services Building, London, ON, Canada N6A 5C1
 Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
 Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Koren

Review Number: 15556

Review Date: July 28, 2009

Protocol Title: CYP2D6 screening for Adverse Drug Reactions to Codeine in Breast Milk

Department and Institution: Paediatrics, University of Western Ontario

Sponsor: CIHR-CANADIAN INSTITUTE OF HEALTH RESEARCH

Ethics Approval Date: July 28, 2009

Expiry Date: December 31, 2012

Documents Reviewed and Approved: Study Methods, Advertisement, Letter of Information and Consent (ver. July 14, 2009)

Documents Received for information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- all adverse and unexpected experiences or events that are both serious and unexpected;
- new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

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Appendix 6: Ethics approval for tonsillectomy clinical trial (UWO, HSC, McMaster)



Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Gideon Koren
 File Number: 102501
 Review Level: Full Board
 Approved Local Adult Participants: 0
 Approved Local Minor Participants: 0
 Protocol Title: Investigating the safety morphine and ibuprofen in young children after adenotonsillectomy for obstructive sleep apnea (REB# 18958)
 Department & Institution: Schulich School of Medicine and Dentistry/Schulich School of Medicine & Dentistry, Western University
 Sponsor: Canadian Institutes of Health Research

Ethics Approval Date: August 01, 2012
 Ethics Expiry Date: December 31, 2013

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Letter of Information & Consent	Version 2.1	
Western University Protocol	Including all instruments listed in section 8.1.	
Sub-Study Letter of Information & Consent	Version 2.1	
Assent	Version 1.0	

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[Redacted Signature]

Signature

Ethics Officer to Contact for Further Information

[Redacted Contact Information]

This is an official document. Please retain the original in your files.

The University of Western Ontario
 Research Development & Services
 Support Services Building, Suite 5150 • London, Ontario • CANADA - N6A 3K7
 PH: 519-661-2161 • F: 519-661-3907 • www.uwo.ca/research



THE HOSPITAL FOR
SICK CHILDREN

RESEARCH ETHICS BOARD

February 03, 2014

Dr. Gideon Koren
Clinical Pharmacology & Toxicology
The Hospital for Sick Children

Dear Dr. Koren:

Your study "Investigating the safety of morphine and ibuprofen in young children after adenotonsillectomy for obstructive sleep apnea"

REB File No.: 1000033184 – January 2014 Reapproval

On behalf of the REB, I am writing to confirm that the above noted study was re-approved by the REB for one year ending in January 2015. The REB approved continuing review at level 2E. Reapproval is granted only for the purposes of retrospective data collection and analysis. Prospective recruiting of subjects must cease as per the DSMB report dated January 31, 2014. As necessary, the Clinical Research Office will be contacting you to arrange follow-up.

Please note that, in accordance with the Personal Health Information Protection Act of Ontario, you are responsible for adhering to all conditions and restrictions imposed by the REB governing the use, security, disclosure, return and disposal of the research subjects' personal health information. You are also responsible for reporting immediately any privacy breaches to the REB Chair and to Janice Campbell, the Sick Kids privacy officer.

Yours truly,



Co-Investigator(s): Lauren Kelly, Doron Sommer, Jonathan Maclean

555 University Ave
Toronto, Ontario
Canada M5G 1X8

www.sickkids.ca



RESEARCH ETHICS BOARD

McMaster
University

REB Office, 293 Wellington St. N., Suite 102, Hamilton, ON L8L 8E7
Telephone: 905-521-2100, Ext. 42013
Fax: 905-577-8378

Research Ethics Board Membership

Suzette Salama PhD
Chair/Ethics Representative
Donald Arnold MD, MSc FRCP(C)
Hematology & Thromboembolism
Uma Athale, MBBS, MD, M.Sc. FRCPC
Pediatric Hematology/Oncology
Mary Bedek CCHRA (C)
Privacy Officer
Joseph Beyene PhD
Clinical Epidemiology & Biostatistics
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Orthopedic Surgery David Clark MD
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Pediatrics
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Clinical Epidemiology & Biostatistics
Kathryn Webert MD
Transfusion Medicine
Andrew Worster MD
Emergency Medicine
Deborah Yamamura MD, B.Sc. Hons.
Pathology & Molecular Medicine
Ed Younglai PhD
Obstetrics/Gynecology

May 28, 2012

PROJECT NUMBER: 12-204

PROJECT TITLE: Investigating the Safety of Morphine and
Ibuprofen in Young Children after
Adenotonsillectomy for Obstructive Sleep
Apnea

PRINCIPAL INVESTIGATOR: Dr. D. Sommer

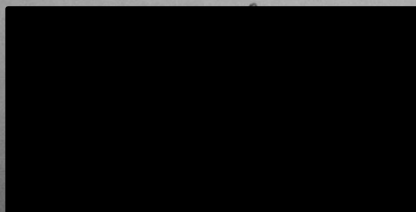
This will acknowledge receipt of your letter dated May 1, 2012 which enclosed revised copies of the Information/Consent Form, Application Form, Parent Data Collection Sheet and the Protocol along with response to the additional queries of the Board for the above-named study. We have noted the change in study title as you have decided to remove codeine from the study. These issues were raised by the Research Ethics Board at their meeting held on April 17, 2012. Based on this additional information, we wish to advise your study has been given **final** approval from the full REB. The submission, Study Protocol version 2.0 dated May 1, 2012 including the Information/Consent Form and Genetic Information/Consent Form, both versions 2.1 together with Assent Form version 1.0; Demographic and Health Information Sheet, and Parent Data Collection Sheets, both versions 2.1 dated May 1, 2012; Objective Pain Scale and Visual Analog Scale, both versions 1.0 dated March 22, 2012 were found to be acceptable on both ethical and scientific grounds. **Please note** attached you will find the Information/Consent Forms and Assent Form with the REB approval affixed; all consent forms/assent forms used in this study must be copies of the attached materials.

We are pleased to issue final approval for the above-named study for a period of 12 months from the date of the REB meeting on April 17, 2012. Continuation beyond that date will require further review and renewal of REB approval. Any changes or revisions to the original submission must be submitted on an REB amendment form for review and approval by the Research Ethics Board.

The Hamilton Health Sciences/McMaster Health Sciences Research Ethics Board operates in compliance with and is constituted in accordance with the requirements of: The Tri-Council Policy Statement on Ethical Conduct of Research Involving Humans; The International Conference on Harmonization of Good Clinical Practices; Part C Division 5 of the Food and Drug Regulations of Health Canada; and the provisions of the Ontario Personal Health Information Protection Act 2004 and its applicable Regulations.

PLEASE QUOTE THE ABOVE-REFERENCE PROJECT NUMBER ON
ALL FUTURE CORRESPONDENCE

Sincerely,



Appendix 7: Clinicaltrials.gov registration for tonsillectomy study

Investigating the Safety of Post-surgical Analgesics in Children With Obstructive Sleep Apnea

[Reset](#)
[close](#)

This study is currently recruiting participants.
Verified May 2013 by Hamilton Health Sciences Corporation

Sponsor:
Hamilton Health Sciences Corporation

Collaborators:
 The Hospital for Sick Children
 University of Western Ontario, Canada

Information provided by (Responsible Party):
 Doron Sommer, Hamilton Health Sciences Corporation

ClinicalTrials.gov Identifier:
NCT01680939

First received: September 4, 2012
 Last updated: May 24, 2013
 Last verified: May 2013
[History of Changes](#)

Full Text View

Tabular View

No Study Results Posted

[Disclaimer](#) [How to Read a Study Record](#)

Purpose

Every year thousands of young children with **obstructive sleep apnea** undergo surgery which requires them to be prescribed pain medication. The current standard in North America is administration of opioids, mainly codeine or morphine; however in many areas of the world including Canada, nonsteroidal anti-inflammatory medications such as ibuprofen are used. Some North American surgeons are uncertain regarding the potential of ibuprofen to increase bleeding following surgery. The results of research studies have been inconclusive overall. Due to recent codeine fatalities in children following adenotonsillectomy, codeine has been removed from the formulary at many Pediatric institutions. Some surgeons have begun to use oral morphine as an alternate to codeine, which necessitates the need to find safe alternative analgesics in this treatment group. The primary objectives of this study is to assess the safety(1) and efficacy (2) of morphine and ibuprofen in children with sleep apnea. An interim analysis will be conducted after recruitment of 70 patients, to monitor both safety and efficacy

Appendix 8: Ethics approval for oral morphine weaning chart review

Office of Research Ethics
The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 5C1
Telephone: (519) 861-3036 Fax: (519) 860-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Western Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Mr. D. Knoppert
Review Number: 16131E **Review Level:** Expedited
Review Date: April 30, 2009

Protocol Title: A comparison of in-hospital vs. at home opioid weaning in newborns
Department and Institution: Pharmacy, St. Joseph's Health Care London
Sponsor: Hollingsworth Scholarship
Ethics Approval Date: May 05, 2009 **Expiry Date:** May 31, 2010

Documents Reviewed and Approved: UWO Protocol

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/CH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 4 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- all adverse and unexpected experiences or events that are both serious and unexpected;
- new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

[Redacted Signature] Chair of HSREB: Dr. Joseph Gibert

UWO HSREB Ethics Approval - Initial
© 2008-07-01 16131E/16131E/16131E Initial 16131E



Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. David Knoppert

Review Number: 16131E

Review Level: Delegated

Approved Local Adult Participants: 50

Approved Local Minor Participants: 0

Protocol Title: A comparison of in-hospital vs. at home opioid weaning in newborns

Department & Institution: Paediatrics, University of Western Ontario

Sponsor:

Ethics Approval Date: March 16, 2011

Expiry Date: December 31, 2013

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Change in Study Personnel	Lauren Kelly will be doing data collection	
Revised UWO Protocol	Revised study end date - December 31, 2013.	

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research

Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices Consolidated Guidelines, and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

Curriculum Vitae

Lauren Elyse Kelly

EDUCATION:

The University of Western Ontario, London, Canada (2010-2014)

Ph.D. Candidate in Clinical Pharmacology

Thesis: "Identifying risk factors for opioid morbidity and mortality in young children"

The University of Birmingham, Birmingham, England (2008-2009)

M.Sc in Human Toxicology

Thesis: "Neonatal central nervous system depression following exposure to oxycodone during lactation"

The University of Western Ontario, London, Canada (2004-2008)

BMSc Specialization Medical Sciences

Concentration: Pharmacology & Toxicology, Biochemistry

AWARDS:

- Best Abstract: Research in Breastfeeding, American Academy of Pediatrics (2013)
 - PhD Studentship, Canadian Pharmacogenomic Network for Drug Safety (2011-2013)
 - Queen Elizabeth II Graduate Scholarship in Science and Technology (2012-2013)
 - Research Award, University of Western Ontario (2010-2014)
 - Schulich Graduate Student Award, University of Western Ontario (2010-2014)
 - Postgraduate Ambassador, University of Birmingham (2008-2009)
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RESEARCH EXPERIENCE:

University of Western Ontario, London, Ontario (2010- present)

Clinical Research Coordinator

Collect informed consent and all data points for several research studies. Responsible for all administrative and ethical proposals as well as study design and data analysis.

Rehabilitation Solutions, Toronto Western Hospital, Toronto, Ontario (2010)

Clinical Research Associate

Duties included meeting with patients and collecting a best possible medication history for the functional restoration and chronic pain program. Data was collected, analyzed and professionally presented to investors.

TEACHING EXPERIENCE:

Teaching Assistant –**Mammalian Physiology 3130Y** (2010 – 2014)

Responsible for teaching in the third year undergraduate physiology laboratory at the University of Western Ontario

Teaching Assistant –**Meds IV Diagnostics and Therapeutics** (2010 – 2014)

Responsible for the design and implementation of a fourth year clinical pharmacology course to prepare students for residency placements

PUBLICATIONS:

- Kelly LE, Knoppert D, Koren G. Pharmacogenomic predictors of neonatal abstinence syndrome - correlation with length of stay. *Therapeutic Drug Monitoring* 2013: in press
- Lam J, Kelly LE, Matok I, Ross CJ, Carleton B, Hayden MR, Madadi P, Koren G. Putative association of ABCB1 2677G>T/A with oxycodone- induced central nervous system depression in breastfeeding mothers. *T Therapeutic Drug Monitoring* 2013; 35(4): 466-472
- Kelly LE, Chaudhry SA, Rieder MJ, 't Jong G, Moretti ME, Lausman A, Ross C, Berger H, Carleton B, Hayden MR, Madadi P, Koren G. A clinical tool for reducing Central Nervous System depression among neonates exposed to codeine in breast milk. *PLoS-One* 2013; 8 (7): e70073
- Kelly LE, Rieder M, Brigdman-Acker K, Lauwers B, Madadi P, Koren G. Are infants exposed to methadone *in utero* at an increased risk for mortality? *J Popul Ther Clin Pharmacology* 2012: 19 (2) e160-166
- Kelly LE and Madadi P. Is there a role for Therapeutic Drug Monitoring with Codeine? *Therapeutic Drug Monitoring* 2012. Accepted March 10, 2012; 34: 249-256
- Kelly LE, Poon S, Madadi P, Koren G. Neonatal exposure to benzodiazepines during breastfeeding. *The Journal of Pediatrics* 2012; 161 (3): 448-457
- Kelly LE, Rieder M, van den Anker J, Malkin B, Ross C, Neely M, Carleton B, Hayden M, Madadi P, Koren G. More codeine fatalities after tonsillectomy in North America. *Pediatrics* 2012; 129 (5) e1343-1347
- Lam J, Kelly LE, Ciszowski C, Landsmeer M, Nauta M, Carleton B, Hayden M, Madadi P, Koren G. Central Nervous System Depression of Neonates Breastfed by Mothers Receiving Oxycodone for Postpartum Analgesia. 2011. *J Peds* 2012; 160 (1): 33-37
- Kelly LE, Rieder M, Veenstra F, et al. Pharmacogenetics of fatal paediatric obstructive sleep apnea cases in response to codeine: additional cases. *J Popul Ther Clin Pharmacol* 2011; 18(2):e345.

PRESENTATIONS:

- Kelly LE, Chaudry SA, Rieder MJ, 'tJong G, Moretti ME, Lausman A, Ross C, Berger H, Carleton B, Hayden MR, Madadi P, Koren G. A clinical tool for reducing Central Nervous System depression among neonates exposed to codeine in breast milk. American Academy of Pediatrics, Orlando, Florida. October 28, 2013
- Kelly LE. Risks of using codeine for maternal pain during breastfeeding. 8th Annual Ivey Symposium. London, Ontario, Canada. October 22, 2013

- Kelly LE. Opioid pharmacology and toxicogenetics. Guest Lecturer Pharmacology 4660. Western University, London, Ontario, Canada. October 21, 2013
- Kelly LE, Chaudry SA, Rieder MJ, 'tJong G, Moretti ME, Lausman A, Ross C, Berger H, Carleton B, Hayden MR, Madadi P, Koren G. A clinical tool for reducing Central Nervous System depression among neonates exposed to codeine in breast milk. Pediatrics Research Day, London Health Sciences Centre. London, Canada. May 29, 2013
- Kelly LE. Investigating the safety of morphine and ibuprofen in children following adenotonsillectomy for obstructive sleep apnea. Canadian Pharmacogenomic Network for Drug Safety Scientific Advisory Board Trainee Presentation. Vancouver, Canada. April 10, 2013
- Kelly LE, Chaudry SA, Rieder MJ, 'tJong G, Moretti ME, Lausman A, Ross C, Berger H, Carleton B, Hayden MR, Madadi P, Koren G. An intervention study for reducing Central Nervous System depression among neonates exposed to codeine in breast milk. London Health Research Day, London, Canada. March 19, 2013
- Kelly LE. Methadone use in pregnancy- pharmacogenomic considerations. 7th Annual Ivey Symposium. London, Ontario, Canada. October 16, 2012
- Kelly LE. Pharmacogenomics of fatal opioid toxicity in young children. Guest Lecturer Pharmacology 4660. Western University, London, Canada. September 10, 2012.
- Kelly LE, Poon S, Madadi P, Koren G. Neonatal exposure to benzodiazepines during breastfeeding. Canadian Society of Pharmacology and Therapeutics Poster Presentation. Toronto, Canada. June 12, 2012
- Kelly LE, Poon S, Madadi P, Koren G. Neonatal exposure to benzodiazepines during breastfeeding. London Health Research Day, London, Canada. March 20, 2012
- Kelly LE. Genetic risk factors for pediatric opioid morbidity and mortality. Canadian Pharmacogenomic Network for Drug Safety Scientific Advisory Board Trainee Presentation. Vancouver, Canada. November 14, 2011
- Kelly LE, Rieder M, van den Anker J, Malkin B, Ross C, Neely M, Carleton B, Hayden M, Madadi P, Koren G. More codeine fatalities after tonsillectomy in North America. Stevenson Lecture and Research Day. London, Canada. November 8, 2011
- Kelly LE, Rieder M, Veenstra F, et al. Pharmacogenetics of fatal pediatric obstructive sleep apnea cases in response to codeine: additional cases. 2011 annual meeting of the Canadian Society of Pharmacology and Therapeutics; Multidisciplinary Approaches to Modern Therapeutics: Joining Forces for a Healthier Tomorrow. Montreal, Canada. May 26, 2011.
- Kelly LE. Opioid Narcotics Use During Breastfeeding. 5th Annual Ivey Symposium. London, Ontario, Canada. October 12, 2010

MEMBERSHIPS:

- Canadian Pharmacogenomic Network for Drug Safety
 - Canadian Society of Pharmacology and Therapeutics
 - American Academy of Pediatrics
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OTHER:

- Senior Reviewer – Journal of Clinical Pharmacology and Population Therapeutics Trainee Section (2013- present)
- Western University Department of Physiology and Pharmacology Graduate Student Council Secretary (2013-present)
- Let's Talk Science Volunteer (2010-present)
- Reviewer – Journal of Obstetrics and Gynecology (2012)
- CIHR - Institute of Human Development, Child and Youth Health Summer Institute Participant (2011)
- Scholar's Elective Mentor (2010-2011)